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THE RÔLE OF THE CELLS OF SCHWANN IN THE FORMATION OF TUMORS OF THE PERIPHERAL NERVES *

PERCIVAL BAILEY, M.D., AND JESS D. HERRMANN, M.D.

(From the Division of Neurology and Neurosurgery, University of Chicago Clinics, Chicago, Ill.)

The origin of tumors of the peripheral nerves has long been a matter of controversy. Two schools of thought are still opposed, the one deriving these tumors from the connective tissue, the other from the cells of Schwann. The former opinion goes back to von Recklinghausen,[59] the latter is usually derived from Verocay,[58] although he was not the first to propose a schwannian origin for these tumors. At the present time the connective tissue hypothesis is defended by Penfield [37] and the schwannian theory by Masson.[29] This controversy had never seriously engaged our interest until recently the hazard of the clinic placed in our hands complete autopsy material from 2 remarkable cases of multiple tumors of the nervous system which seemed to promise opportunities for forming an opinion based on personal investigation. Our observations on this material form the primary basis of this study.

METHODS

The subjects of this study were two unrelated girls, 15 and 16 years of age respectively at the time of death. Complete autopsies were performed in each instance; 4½ hours after death in 1 case (B. S.), and 12 hours postmortem in the other (E. N.). There were also surgical specimens from each case fixed immediately at the moment of removal at operation. In the 1st case (B. S.), numerous fragments of various nerves and nerve roots were fixed

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in formalin, formol-Zenker, Orth's, Bouin's, and Zenker's fluids, absolute alcohol, ammoniated alcohol, and Domenici's and Laguesse's (formula J) fixatives and Weigert's Gliabeize. The material being ample, transverse and longitudinal sections from each nerve were made and stained by the following methods: Cajal's reduced silver, Nageotte's hematoxylin, Herxheimer's scarlet red, Freeman's silver method for nerve fibers, Mallory's phosphotungstic acid hematoxylin, Loyez's method for myelin, Weil's method for myelin, Laidlaw's and Perdrau's methods for connective tissue, van Gieson's, Foot's silver method for connective tissue on Zenker-fixed material, the method of Gros-Bielschowsky, cresyl violet, neutral ethyl violet-orange G, hematoxylin and eosin, mucicarmine, Bodian's method for nerve fibers, Ranson's method for unmyelinated nerve fibers, Regaud's and Cowdry's methods for mitochondria, the methods of Jakob, Doinikow, and many others in certain instances. The brain and spinal cord were fixed by immersion in formalin after removal of blocks from the hypothalamic tumor and from the spinal cord for fixation in Zenker's fluid, alcohol and formalin bromide. In addition, preparations from the skin were made with Bloch's dopa reaction. The brain and spinal cord were later studied after staining with the usual methods used by neuropathologists following fixation in formalin (10 per cent).

In the 2nd case, certain peripheral nerves, notably the ulnar and median nerves in the arm, the lower cord of the brachial plexus, the upper cord of the lumbar plexus, the vagus and sympathetic trunks in the lower thorax and the ilioinguinal nerve, were removed and fixed in the same manner for comparison with those of the 1st case, although they were not grossly abnormal. The brain and spinal cord were fixed by immersion in 10 per cent formalin together with the associated tumors of meninges and nerve roots. Tumors from the upper cervical region and intracranial meninges, however, had already been removed at operation and studied after immediate fixation in 10 per cent formalin, Zenker's fluid and the formalin bromide solution of Cajal. In addition, abundant material from other cases of all sorts of tumors of the central and peripheral nervous systems, collected over many years in the pathological laboratories, were used for comparative study. We also repeated the classical experiments of Nageotte and produced tumors in rabbits by autotransplantation of peripheral nerves.

We finally made numerous preparations from normal nerves by all these methods for comparison.

We must confess at the outset that we did not obtain profitable results with any of the methods of Nageotte on our pathological material. Perhaps the material was unsuitable, perhaps our lack of familiarity with the methods was at fault. The best preparations for axis cylinders were obtained with the methods of Bodian [7] and Freeman.[19] The former method gave consistently usable preparations; the latter was more fickle, impregnating often neurokeratin, but when it did give a specific impregnation the preparations were perfect for our purposes since the connective tissue was completely colorless and could be counterstained by the method of van Gieson. Sharply specific preparations of neurokeratin we obtained with Mallory's phosphotungstic acid hematoxylin on formalin-fixed paraffin sections by omitting to treat the sections with oxalic acid and permanganate. In these preparations any fragment of a myelin sheath stood out sharply blue against a uniform salmon pink background. They were found very difficult to photograph, but perfect for study. Many excellent methods for connective tissue were available, both for staining (Masson, Mallory) and for impregnation (Perdrau, Laidlaw, Foot). The method of Foot, when used on Zenker-fixed material, impregnated much more than the methods of Laidlaw and Perdrau when used on formalin-fixed material from the same region. The sections prepared by Masson's trichrome method on Bouin-fixed material gave beautiful preparations which we found, however, very difficult to photograph, so that for this purpose we were obliged often to have recourse to the ancient but excellent method of van Gieson. Often we wished we might have colored drawings made, but the expense was too great and besides we wished, if possible, to record our observations by actual photographs rather than by drawings, necessarily to a variable extent interpretative. If the number of photographs be found excessive, we can only plead our belief that one good photograph is more useful than many pages of verbal description.*

Finally, we regret the lack of any utilizable specific method for

* We must here record our indebtedness to our photographer, Mr. Francis T. Harmon, without whose skill with filters many of our observations could not have been adequately recorded by photographic means.

staining or impregnating the cells of Schwann. After repeated but fruitless efforts with the methods devised by Cajal, Nageotte, Dockrill and others, we abandoned them in despair. A specific method for these cells would immediately resolve many uncertainties and obviate much indirect argumentation, however plausible. We will proceed, therefore, immediately with the description of our observations, made with the admittedly imperfect methods at our disposal, and afterwards try to determine how far any valid conclusions may be drawn from them concerning the rôle of the cells of Schwann in the formation of tumors of the peripheral nerves.

Case Reports

Case 1. *Clinical History:* B. S., a girl aged 12 years, was referred to the clinic on Feb. 5, 1929 (Unit No. 9648), complaining of failing vision. (Previously briefly reported from a clinical standpoint by Bailey, Intracranial Tumors, Case 36.)

In 1927 it was noted at school that she could not read from the blackboard. She had complained of backache a year previously and had worn a sacro-iliac belt for 6 months, but had otherwise been well except for repeated attacks of asthma. The family history was unimportant except that the father had over his body numerous manifestations of generalized neurofibromatosis. The mother, one brother and one sister were free from such malformations.

The patient was slender but healthy in appearance. On the skin were numerous brownish patches varying in size from 1 to 3 cm. in diameter. There were also numerous, small, violet colored soft elevations about 1 cm. in diameter. Small, firm subcutaneous nodules could be felt along the course of the right median, right supraorbital and left greater auricular nerves. Over the anterior aspect of the right ankle was a boggy mass 5 cm. in diameter in which firm discrete nodules could be felt. The optic nerve heads were normal but vision was much reduced. No other abnormality of the nervous system was found. A diagnosis of generalized neurofibromatosis complicated by glioma of the optic chiasm was made. An X-ray of the head was made in an attempt to see the optic canals but it was not successful.

The child was taken to another clinic where, on March 4, 1929, a right transfrontal exposure was made, disclosing a tumor involving the chiasm and both optic nerves. The nodule in the right cubital fossa was also exposed and was found to involve the median nerve so firmly that it could not be removed.

She returned on July 5, 1929. The vision at this time was found to be R = 0.1 and L = 0.6. There were bizarre temporal defects in each visual field. The fundi appeared normal.

Between July 9, 1929, and Jan. 29, 1930, she was given roentgen radiation directed toward the optic chiasm through the temporal regions. The visual fields did not change, the acuity very little. On Oct. 18, 1930, the right acuity was 0.4–2 and left 0.6–2. On Jan. 6, 1930, an X-ray of the optic canals demonstrated enlargement of both of them, the right being slightly larger than the left. On April 3, 1931, the basal metabolism was found to be −11

per cent. She weighed at that time 63.4 kg. and was 162 cm. in height. On Nov. 27, 1932, she had grown slightly stouter. The optic discs were slightly pale, but seemed otherwise unchanged. On May 29, 1933, she came complaining of attacks of pain behind and under the left ear. Her speech was a little thick and there was some atrophy of the right half of the tongue. She then weighed 66.2 kg. and was 162 cm. high. She had been menstruating regularly for 2 years. Vision was unchanged. On June 3, 1933, audiometer tests demonstrated normal hearing in both ears, and caloric tests showed both labyrinths to be functioning normally.

She was admitted to the hospital on Oct. 31, 1933 because of increasing difficulty in swallowing. At this time she weighed only 56.2 kg. and her height was 164 cm. The basal metabolic rate was −3 per cent. The cutaneous manifestations previously noted had not changed much except that numerous peripheral nerves could be palpated under the skin. The breasts were small. The bodily hair was normally abundant. The menses were not disturbed. There had never been any polyuria. The visual acuity and visual fields were as previously noted in October 1930, but the optic discs looked paler. The pupils were round, equal, 5 mm. in diameter and reacted well to light. There was a nystagmus on looking to right and left, slower and coarser to the left. Convergence was fair. Hearing was normal in both ears to audiometer, and normal caloric responses were obtained in each ear. External ocular movements were normal. Motor and sensory fifth nerves were intact bilaterally. There was possibly slight weakness of the seventh nerve on the right side. Sensation over the right side of the pharynx was diminished. The right palatal and pharyngeal muscles were weakened and the right vocal cord paretic. The sternomastoid and trapezius muscles seemed of normal strength bilaterally. The right half of the tongue was atrophied. Speech was slurred and the patient was much troubled by accumulation of mucus in the throat which she could swallow only with difficulty. The muscles of the right arm and leg seemed slightly weaker and at times a dorsal plantar response was obtained on the right side. No defect of general sensation was demonstrated over the body. The gait was slightly unsteady and she fell constantly to the left and backward in Romberg's position. There was no definite incoordination of the extremities.

A diagnosis of neurofibroma of the right twelfth nerve, compressing the ninth and tenth nerves and the bulb, was made. On Nov. 4, 1933, an attempt was made to expose the posterior fossa, but it had to be abandoned because of inability to flex the head forward so as to expose the region. An attempt to anesthetize with ether in the hope that relaxation of the muscles would enable the head to be flexed forward resulted in cessation of respiration. The patient afterward complained of pain in the back and right gluteal region. It was planned to make a roentgenogram of the spine, but she continued to have difficulty with breathing and suddenly at 5:00 A.M. on Nov. 7, 1933 she died.

Postmortem Examination

Autopsy was performed 4½ hours postmortem. Acute distention of the heart, a menstruating uterus, and bilateral follicular cysts of the ovaries were the insignificant findings in the viscera,

except for the alterations of the nerves. The vagus nerves were enlarged from the foramina jugularia to their finest ramifications. In fact, every nerve in the body, so far as could be seen, was enlarged in irregular fashion, often looking like a string of beads. The vagus nerves at the level of the bifurcation of the trachea measured approximately 1 cm. in diameter. The phrenic nerves also varied from 0.5 to 1 cm. in diameter. The sympathetic chains were in places as much as 3 cm. in diameter. It is futile to describe all the nerves; down to their finest ramifications they were clearly visible, appearing as though injected and distended by some milky fluid. The nerves of the extremities were generally enlarged and contained fusiform swellings which reached as much as 3 cm. in diameter.

When the brain was removed the convexity appeared practically normal; perhaps the convolutions were slightly flattened. There were no tumors of the meninges. The olfactory bulbs were normal. Both optic nerves and the optic chiasm were greatly enlarged. In the angle between the bulb, pons and cerebellum on the right side was a firm nodular tumor 3 cm. in diameter. It appeared to arise from the twelfth nerve and there was an extension of the tumor through the canalis hypoglossi which was transected when the brain was removed. The tumor indented the bulb deeply and to a less extent the cerebellum and pons. The third, fourth, fifth and sixth cranial nerves were normal. The seventh, eighth, ninth, tenth, eleventh and twelfth cranial nerves on the left side were normal near their origin, but the ninth, tenth, eleventh and twelfth nerves swelled suddenly just before passing through their foramens of exit. On the right side the seventh and eighth nerves were elongated because of distortion by the tumor of the twelfth nerve, but were otherwise normal. The ninth, tenth and eleventh nerves swelled suddenly at their foramens of exit, as did those on the left side. The right twelfth nerve could not be identified, but the tumor before mentioned projected through the canalis hypoglossi.

On median sagittal section of the brain the tumor of the optic chiasm was seen to obliterate the infundibulum, extending from the mammillary bodies to the anterior commissure. On cross section of the cerebrum the tissue appeared normal. No tumors, macrogyria or other abnormalities were found. In the cerebellum, how-

ever, there was found in the left hemisphere, just back of the dentate nucleus, a grayish tumor 1.5 cm. in diameter.

All of the roots of the spinal nerves were more or less swollen, some of them bearing intraspinal tumors of considerable size. The third thoracic nerve on the left side bore a tumor 1.5 cm. in diameter. The cauda equina looked like a cluster of grapes, all of the roots bearing nodules of tumor in strings. The filum terminale was slightly enlarged, but had no gross tumorous nodules. These tumors were clearly arising from the roots of the nerves and not from the meninges.

Microscopic Examination

The soft subcutaneous mass above the right ankle proved on microscopic examination to be a lipoma. It was not otherwise remarkable except that the nerve trunks in the connective tissue septums were grossly enlarged. The nerve fibers were few and either widely separated from one another or collected into a small portion of the cross section by a great overgrowth of the supporting tissue. This overgrowth consisted mainly of a great number of spindle cells with scanty cytoplasm and elongated dense nuclei. The cells were accompanied by myriads of reticulin fibrils. In other nerves the cells of the overgrowth were thicker, with sometimes abundant cytoplasm and larger oval nuclei. This supporting tissue had in places undergone a degenerative change resulting in a finely granular precipitate between the cells.

The pigmented areas of the skin were not remarkable except that there was an almost unbroken row of cells in the stratum germinativum clearly demonstrated by Bloch's dopa reaction.

The purple, soft subcutaneous lesions had undergone extensive degeneration. Between the cells was permeated everywhere a homogeneous glassy material which stained faintly blue with hematoxylin and also with aniline blue. In this homogeneous jelly-like material floated various cells, strands of collagen and blood vessels. In areas that had not undergone this degeneration could be seen cells with considerable cytoplasm, some of them very elongated, others round, others club shaped, lying in irregular masses and separated by a variable amount of reticulin or collagen. These cells resembled very much the cells in nevi described by Masson. The homogeneous material seemed to arise by degeneration of these cells.

The optic nerves consisted mainly of a dense gliosis divided into funiculi by septums of connective tissue extending inward from the sheath. In the outer funiculi were a few myelinated nerve fibers, particularly in the inner margin of the left nerve and the upper and inner margins of the right nerve. Comparison with Freeman preparations was convincing that many more nerve fibers persisted than the myelin sheath preparations would lead one to suppose, a few fibers being found even in the innermost funiculi. The glial scar was composed of piloid astrocytes usually elongated in the long axis of the nerve, although there was a condensation of neuroglial fibrils often along the septums of connective tissue which ran at various angles to the main direction of the fibers of the gliosis.

The optic chiasm continued without interruption into the tumor of which it formed the anterior margin. The tumor was composed principally of large bipolar spongioblasts. It was surrounded laterally by a dense gliosis containing concentric masses of calcification, and passed over anteriorly by gradual transition into the gliosed optic nerves. In the anterior margin of the tumor there were many nerve fibers both myelinated and unmyelinated. There was no fatty degeneration either in the optic nerves, chiasm or tumor. The gliosis around the tumor contained great numbers of the so-called Rosenthal fibers. Within the brain was found, in addition to the spongioblastoma of the optic chiasm and hypothalmus, a round subcortical tumor in the cerebellum, 1.5 cm. in diameter, having the typical structure of a protoplasmic astrocytoma. Also, in the molecular layer of the cerebellum were nodules of abnormal cells seeming, from their reactions to impregnation methods, to be composed of both neuroglial and microglial cells. These nodules had no relation to blood vessels and were numerous in the molecular layer of the cerebellum. No such abnormal accumulations could be found in the cerebral cortex, but often, particularly in the frontal lobes, were seen abnormal accumulations of oligodendroglia and neuroglia along the walls of small blood vessels. In the spinal cord, no pathological alterations of the interstitial cells were found, but often there was a considerable collection of pathological cells at the point of exit of the anterior spinal roots which extended as much as 2 mm. within the cord. These cellular clumps formed numerous fibrils of reticulin

and appeared like small neurinomas. The cerebral cyto-architectonics seemed to us to be normal and the nerve cells and nerve fibers unaltered. The intracranial leptomeninges and blood vessels were microscopically normal.

The tumor arising from the second cervical nerve root was 2 cm. in diameter. There was very little fatty degeneration in the tumor, only a few fat-containing macrophages being seen along the walls of the vessels. Throughout the tumor were nerve fibers, usually in groups but often widely scattered. Some of these had no myelin sheaths, but most were myelinated. These fibers were not accumulated at the periphery of the tumor. There was a thin capsule of the tumor composed of parallel strands of collagenic connective tissue. The cells of the neoplasm had very little cytoplasm. Many of them were elongated, with their long axes parallel to the nerve fibers. Whenever nerve fibers were cut longitudinally in the sections the tumor cells were also cut longitudinally and *vice versa*. The tumor cells were accompanied by vast numbers of delicate collagenic fibrils. Their nuclei were the typical, crenated, wrinkled fibroblastic nuclei so often drawn by Maximow. Where the nerve fibers were cut transversely it could be seen that these collagenic bands collected around the nerve fibers, but many such collections contained no nerve fibers. The tissue was very loose, being dissociated apparently by edema. There were great numbers of loose cells of rounded or slightly elongated form which were free of any association with the nerve fibers or collagenic bands. Many of these looked like lymphocytes but most had larger nuclei resembling those of ordinary connective tissue cells. Many were undoubtedly macrophages and had fine granules of fat in their cytoplasm. These loose cells ran the entire gamut of Maximow's polyblastic series. Very few cells could be seen that had any resemblance to Schwann cells. There were a few ganglion cells present. Some were surrounded by capsular cells, but others by what appeared to be cells very similar to the other tumor cells forming abundant collagen. A few fenestrated cytoplasmic bands were found containing unmyelinated nerve fibers, undoubtedly Remak fibers. Rarely a collagenic bundle would contain a fat nucleated cell without myelin sheath or nerve fiber, possibly a Buengner cord. There was no tendency whatever for the cells to form whorls about the nerve fibers. Blood vessels were scanty, composed of endothelial cylinders surrounded

by a few strands of collagen. Most of the intercellular substances reacted to stains and impregnations similar to collagen. There was very little reticulin and no elastin.

Numerous spinal roots had similar tumors of greater or lesser size. Their structure was so similar that repeated descriptions are unnecessary. Within them were found occasionally formations resembling Wagner-Meissner corpuscles.

The larger tumor on the twelfth cranial nerve was composed of spindle shaped cells with a fair amount of cytoplasm. The nuclei varied from oval forms resembling closely those of ordinary fibroblasts to long sausage shaped forms with denser chromatinic accumulation. These nuclei showed very little tendency to lie in palisades. No mitoses were found. The cells lay in broad interlacing bands. Myriads of reticulin fibrils were laid down between the neoplastic cells running always in the direction of the long axis of the cells. No fatty degeneration was found anywhere in the tumor. No persisting nerve fibers were found.

The left vagus nerve within the cranial cavity measured 4 mm. in diameter. It was dissected down through the jugular foramen and a segment about 1.4 cm. in length was removed. The lower end contained a group of ganglion cells of the ganglion jugulare. These ganglion cells appeared fairly normal. Two types could be distinguished, one group being smaller and darker than the others. The cells contained granular tigroid material and some lipochrome pigment. They were surrounded by capsular cells. The nerve itself was thickened by a great overgrowth of collagenic tissue for the most part. The myelinated nerve fibers were disseminated throughout the cross section, sometimes lying nearer together, at others widely separated. Very few cytoplasmic bands were seen in the center of collagenic accumulations. There seemed to have been very little destruction of the nerve fibers. Around many of the fibers the collagenic bundles were increased. A few areas filled with a loose connective tissue composed of fibroblasts and cells of the polyblastic series were present. These areas seemed very edematous, the cells being widely separated, the polyblasts lying in a loose meshwork of fibroblasts. In other areas the tissue was very dense, thick bands of collagenic fibrils with fibroblastic nuclei dispersed among them lying parallel to the nerve fibers.

The right vagus nerve in its intracranial extent being essen-

tially similar in structure to the left vagus nerve no separate description is necessary.

Along the spinal nerve roots and along the roots of the cauda equina were nodules of various sizes which had the typical structure of neurinomas. They were composed of tenuous spindle cells, running in streams, which formed myriads of delicate reticulin fibrils. Usually these nodules were too large to permit of any opinion concerning their origin, but some were found of small size and these (Fig. 1) could be seen clearly to arise often eccentrically in the roots. One was found (Fig. 2) which unmistakably was developing in the arachnoid. After examining great numbers of sections of the nerve roots, there was no doubt left in our minds that these small neurinomatous nodules arose almost invariably in relation to the perineurium. A few small nodules within the nerve roots had no obvious relation to the perineurium.

The microscopic alterations in the peripheral nerves may be well illustrated by a description of the upper cord of the lumbar plexus. In preparations for nerve fibers and for myelin sheaths, it could be seen that the nerve fibers were dispersed in irregular fashion throughout the cross section of the funiculi, either singly or in groups. At times most of the nerve fibers would be collected into the center of the funiculus, at others largely around the periphery, but usually in irregular fashion throughout. Whenever the nerve enlarged markedly, the nerve fibers obviously diminished in proportion, so that the enlargement of the trunk was not due to increase in number of nerve fibers. Nor were ganglion cells present, so that the enlargements were not due to the formation of ganglions such as cause enlargements of the normal sympathetic chain.

Many of the nerve fibers were normal, others in all stages of degeneration. All of the alterations so well described by Cajal, Nageotte, Jakob and others could be identified in the myelinated nerve fibers; it is unnecessary here to describe them in detail. We will remark only the presence of end stages. Throughout these nerves were found cytoplasmic nucleated cords of the size of a large myelinated nerve fiber. The cytoplasm was stained yellow by van Gieson's and red by Masson's trichrome method. Each such cytoplasmic cord was surrounded by a sheath reacting similar to collagen. These cords were usually isolated but sometimes in groups (Figs. 3, 4, 7, 16). We believe them to be Buengner cords

resulting from hypertrophy of the Schwann cells after the degeneration of myelinated nerve fibers, so well described by Nageotte and recently by Masson. Hereafter such formations, for the sake of brevity, will be referred to by this name without further description. The axis cylinders themselves were found, in these degenerated areas, swollen and fragmented in typical fashion. The unmyelinated nerve fibers were found in clusters, lying in the fenestrated cytoplasm of Remak cells (Figs. 11 and 17). These unmyelinated fibers we found always running parallel (Fig. 10), and in dozens of sections we could never find any anastomoses or divisions of these fibers. Swellings from time to time occurred along their course (Fig. 24), but never did they seem to pass outside the Remak bundles. In degenerated areas the Remak bundles were often greatly hypertrophied, their cytoplasm thickened and the unmyelinated nerve fibers absent (Figs. 13 and 14). Often they were transformed into more or less homogeneous masses which stained like collagen but more faintly (Figs. 21 and 22). Around each myelinated nerve fiber and around each Remak band were numerous fibrils of reticulin or collagen which were impregnated by Perdrau's method (Fig. 20) and stained red by van Gieson's method. These fibrils could be seen clearly in close association with the sheaths of Schwann around the myelin rings and seemed to us to be adherent to the outer surface of the Schwann sheaths rather than within them. These fibrils form the well known Plenk-Laidlaw sheath and were often greatly increased in numbers which, remaining after the disappearance of the nerve fibers, appeared as thick cords of collagenic fibrils. Such accumulations could also be seen about the Buengner cords. When Foot's method was used on Zenker-fixed tissue, the cytoplasm of the Remak bands was impregnated *in toto* (Fig. 19) and in degenerated areas where the Remak bands were hypertrophied they were also stained blue by Masson's trichrome and red by van Gieson's method. It is possible that many of these large fenestrated structures that contained no unmyelinated nerve fibers may have been hypertrophied Buengner cords secondarily subdivided by collagenic partitions as described by Masson so well in amputation neuromas (Figs. 5 and 6).

Between the nerve fibers and associated structures (Buengner cords, Remak bands, Schwann sheaths) in addition to the fibrils

of the Plenk-Laidlaw sheaths there was a vast accumulation of cells and material which seemed to us to be clearly connective tissue in nature. Typical fibroblasts with the wrinkled crenelated nuclei so often drawn by Maximow were omnipresent (Figs. 23 and 26). Often large areas of dense, pure fibroblastic proliferation could be found (Fig. 26). Cells of the polyblastic series were scattered about and bundles of collagenic fibrils twisted and writhed in every direction among the other elements which were often separated widely by the accumulation of a homogeneous or finely granular material staining very feebly by any method and giving only a faint reaction to mucicarmine (Fig. 16). This myxoid edematous material seemed to be formed by degeneration of the interfibrillary connective tissue; the process could be followed in all stages. Nothing resembling a neurinomatous formation was found. Sometimes a condensation of cells occurred at the periphery of a funiculus; usually no distinction could be seen between the connective tissue in the periphery and center of a funiculus so that no definite perineurium could be said to be present. The cells in the periphery had the same fusiform shape as the interfibrillary ones and sometimes seemed to form a syncytium.

The left median nerve measured 1.4 by 1 cm. in diameter in the upper arm. The only fat found in the cross section of the nerve was contained in large fat cells in the epineurium. The epineurium appeared normal, but the rest of the nerve was decidedly abnormal. Many of the funiculi were enormously enlarged. Many funiculi had an easily recognizable perineurium consisting of densely packed collagenic bundles in which were interspersed elongated nuclei, but in most of the funiculi the tissue of the inner surface of the perineurium became looser and proliferated, its interstices filled with a coagulum which often took a bluish tinge in preparations stained with hematoxylin and eosin. This proliferation sometimes occupied as much as two-thirds of the cross section. Usually the separation between the perineurium and the nervous fasciculi was sharp, but in places, and in certain funiculi, there was no distinction between perineurium and endoneurium. In some funiculi this loose connective tissue with the accompanying bluish coagulum permeated throughout the cross section, in others only one-half or a smaller portion of the funiculus was involved. At one point on the inner surface of the perineurium of a large funiculus was a

proliferation of cells that bore a very close resemblance to a neurinoma. Any part of the cross section of a funiculus might be the seat of a proliferation of the endoneurial connective tissue with a great proliferation of reticulin and collagen, the whole grossly swollen apparently by the accumulation of a colloidal material in the interstices. Myelinated nerve fibers were scattered throughout, but there were areas, where the connective tissue proliferation was most intense, in which no nerve fibers were to be found and even in areas where the nerve fibers were most numerous there were septums of connective tissue containing no nerve fibers. Around each myelin sheath was a ring of material giving the reactions of collagen, of greater or less thickness. Usually distinct collagenic fibrils could be made out running in the same direction as the nerve fibers, but often the sheath appeared homogeneous, even when impregnated with silver. Often a large mass of cytoplasm would be found to contain one or more small myelinated fibers and several unmyelinated fibers. Such a mass looked fenestrated on cross section when stained with Foot's method, phosphotungstic acid hematoxylin or the trichrome stain. Many of the fenestrations contained no nerve fibers. Nothing resembling Buengner cords of degenerated myelinated fibers could be identified. The great increase in size of the nerve seemed to us to be due clearly to proliferation of the endoneurial and perineurial connective tissue plus a tremendous accumulation of edematous fluid which did not give any reaction in this case for mucin.

In the cubital fossa the median nerve suddenly enlarged to form a swelling 1.8 cm. in diameter in fusiform fashion about 2 cm. in length. In this region the nerve fibers were present only in the extreme periphery of the cross section. The rest of the tumor was made up essentially of a loose connective tissue. The spindle shaped cells associated with bands of collagenic fibrils were widely dissociated by an edematous fluid represented in the sections by a delicate coagulum which in some areas became a homogeneous glossy hyalin. This material did not give the reaction of mucin to mucicarmine or only very faintly so. In the interstices of the collagenic meshwork were loose cells running the entire gamut of the polyblastic series. The nuclei of these cells were typical connective tissue nuclei, wrinkled and crenelated. Here and there one saw homogeneous columns with smaller nuclei which we have

interpreted as sclerosed Remak columns. A few Buengner cords in the periphery of the tumor near the nerve fibers were seen.

The right ulnar nerve in the cubital fossa measured only 7 mm. in diameter. There was no fat except in the epineurium. The structure was in no wise different from that of the median nerve so that a separate description is unnecessary.

The right sympathetic chain was greatly thickened, measuring as much as 5 mm. in diameter even between ganglia; no fat was present except in the epineurium. The ganglion cells with their capsular cells were clearly recognizable (Fig. 25), also the bands of unmyelinated fibers with their accompanying Remak cells. Occasionally a myelinated fiber was found. But these structures made up only a small part of the cross section. The greater part of the increase was due to a proliferation of fibroblasts. In some areas there was no admixture of nervous elements, only fibroblasts and cells of the polyblastic series being present, together with bands of collagen and a great amount of granular and glossy débris which stained faintly for mucin. In some areas the fibroblasts formed very little collagen, in others dense masses of fibrils. Many of the Remak bundles had lost their nerve fibers, especially those widely scattered in the fibroblastic areas, and others had undergone a sclerotic change and been transformed into homogeneous masses which stained rather faintly like collagen.

The right ilioinguinal nerve varied in diameter from 3 to 5 mm. In the thickest parts the cross section was made up almost entirely of fibroblastic proliferation which was continuous with the perineurium without any recognizable transition. The connective tissue was very edematous for the most part, interspersed with hyalin and granular coagulum, and masses of collagenic fibrils. Around the periphery of the cross section a few myelinated nerve fibers and many Remak bundles persisted, some of which were sclerosed and had lost their axis cylinders. The myelinated fibers were often surrounded by dense masses of collagenic fibers running parallel with the nerve fibers.

The greater auricular nerve showed changes similar to those found in the ilioinguinal nerve.

CASE 2. *Clinical History:* E. N., a girl of 13 years, was admitted to the University of Chicago Clinics on May 2, 1934 (Unit No. 103364) complaining of paralysis of the arms and legs.

Some 18 months previous to admission she began to complain of weakness of the right arm and leg. For nearly a year she had had difficulty in starting urine. She complained somewhat of pain in the back of the neck. She never complained of headache. In February, 1934, she began to drag the right foot. She was taken to a hospital where a lumbar puncture was made. After this she grew rapidly worse. The right arm and leg became paralyzed and the left arm and leg progressively weaker. An X-ray of the head demonstrated an irregular patchy erosion of the cranium in the left parietal region, which looked like a syphilitic lesion. In spite of negative Wassermann tests on the blood and cerebrospinal fluid, antiluetic treatment was begun, but the patient grew steadily worse and began to have difficulty in breathing. A biopsy of the lesion in the skull showed it to be due to erosion by a meningeal tumor.

On admission she was a tall, well developed girl, 5 feet, 8 inches high, weighing 152 pounds, with large pendulous breasts. She was unable to sit up, stand or walk. Vision was normal in both eyes. The optic discs were normal and visual fields full. The right pupil was slightly larger and reacted sluggishly to light. The left pupil reacted promptly by direct and consensual stimulation. There was a very fine and rapid nystagmus in each eye on looking to right and left. The external ocular movements were normal. There was a right facial weakness on voluntary movement, of peripheral type. Both sternomastoid muscles were weak; the right more so. The right trapezius muscle was paralyzed; the left was very weak. The tongue was normal; also the palatal and pharyngeal musculature.

The patient could scarcely raise her head from the pillow. No voluntary movement could be made with right arm or leg; the left arm and leg were very weak. All extremities were flaccid but the tendon reflexes were all present except the right ankle jerk. The right knee jerk even seemed exaggerated. There was also a bilateral dorsal plantar response. The abdominal reflexes were present on the left side and absent on the right. There was a sustained patellar clonus on both sides, but a clonus was obtained only at the left ankle. The sensory findings varied greatly at different examinations; most constantly found were astereognosis and inability to recognize letters and numbers in the right palm, and diminution of vibratory sensitivity over the right half of the body. At times a sensory loss to pinprick could be found on the left side with a level about C 3 to C 5.

With the laryngoscope both vocal cords could be seen to move freely. Under the fluoroscope both diaphragms could be seen to move paradoxically. X-ray of the skull demonstrated again the irregular erosion of the left parietal bone, yet the findings indicated that the neurological symptoms were predominantly due to some lesion in the spinal canal above the origin of the phrenic nerves. Lipiodol was injected into the lumbar sac. Queckenstedt's test was positive, the fluid was clear, and two lymphocytes were present. Under the fluoroscope, as the patient was tilted head downward, the oil stopped temporarily at L 1, then moved rapidly upward to C 5. Lipiodol injected into the posterior cistern remained there and would not descend into the cervical canal.

On May 10, 1934, under local anesthesia with novocaine, the posterior fossa of the skull was opened and a laminectomy made of C 1, 2, 3 and 4. This exposure was very difficult because the head could not be flexed forward. When the posterior cistern was opened it could be seen that there was a tumor anterior to the spinal cord extending from the foramen magnum to the lamina of the third cervical vertebra. The spinal cord was stretched like a tent over

its posterior surface. The tumor was exposed by sectioning the first and second cervical nerves on the right side and rotating the cord to the left. The tumor was attached firmly to a broad base anteriorly. It was removed piecemeal and the bed curetted with a sharp curette. The wound was then closed and the head, neck and shoulders immobilized by a plaster cast. There had been no respiratory or other difficulty during the procedure. Microscopically the tumor proved to be a meningioma.

The patient improved gradually until May 15th. The paresis of the left arm and leg was not increased but more definite sensory loss could now be demonstrated below C 4. On May 15th she began to have a septic temperature. Leukocytosis was 16,500. The wound was clean, however, and the fever soon subsided. By May 24th she could flex the right elbow and the diaphragm was moving well on each side. On June 1st she was discharged. At this time she had recovered the ability to move the right arm and leg. The dorsal plantar response was present on the right side but not on the left.

On Oct. 28, 1934, she was readmitted to the hospital. She was then able to walk long distances. The right pupil did not react to strong light directly or consensually. It was slightly larger than the left pupil which reacted both directly and consensually to light. The left pupil reacted to accommodation; the right did not. There was a rapid nystagmus of both eyes on looking to the left and upward; none on looking to the right. The external ocular movements were normal. There was no facial weakness. The sternomastoid and trapezius muscles contracted strongly on each side. All tendon reflexes were present except at the right ankle, somewhat brisker on the right side. There was a dorsal plantar response on the right side only. There was no clonus at ankle or patella. No sensory loss of any kind was detected over the body or extremities. The grasp of the right hand was slightly weaker than that of the left, and its thenar and hypothenar eminences slightly flattened, otherwise the muscles of the extremities seemed symmetrically and normally strong. The patient walked and ran normally but the right leg tired more quickly. Visual acuity of the right eye was 0.8–3 and of the left eye 0.2–1. The right optic disc was slightly redder than normal, flat, with normal cupping and well defined borders; the left optic disc was also slightly redder than normal but the upper and nasal borders were slightly elevated. An X-ray of the skull did not reveal any extension of the parietal lesion. The optic foramens seemed of normal size.

The patient was discharged and returned Jan. 28, 1935, at which time her condition was practically the same. She weighed now 77.5 kg. The Babinski sign on the right side had disappeared. On Feb. 19, 1935, under avertin anesthesia supplemented with ether, the eroded area in the left parietal bone was encircled and the bone infiltrated by tumor was removed with a rongeur. The dura mater was then opened laterally and reflected to the longitudinal sinus. There was only a thin sheet of tumor a few millimeters in thickness inside the dura mater. The dura mater was removed up to the lateral wall of the sinus. Some tumor on the lateral wall was cauterized. In doing so the wall was penetrated. Smart bleeding was stopped with muscle. The scalp was closed over the defect, the dura mater being left open. The tumor was proved by microscopic examination to be a meningioma thoroughly infiltrating the dura mater.

There was subsequently slight weakness of the right hand, and numbness and tingling of the right hand and right side of the lips which increased until

February 22nd. There was also difficulty of expression which began to improve about February 25th and rapidly disappeared. The wound healed well except for one persistent sinus which had not entirely closed when she was discharged on March 27, 1935.

She remained perfectly well until June 28th when she had a generalized convulsion. On July 24th she had a series of 8 convulsions. She was examined on Aug. 27, 1935, at which time the ocular findings were unchanged and the right arm and leg seemed weaker but there was no Babinski sign. She was given phenobarbital, 30 mg. thrice daily. On Aug. 25, 1935, there was a definite right hemiparesis with positive Babinski sign. There was also a definite forced grasping in the right hand. There had been no more convulsions.

She was readmitted to the hospital on Oct. 10, 1935, complaining that she could not think well. The left parietal area was bulging but soft. The left optic disc was elevated about 1 D. The ocular findings were otherwise unchanged. There was a right hemiparesis with positive Babinski sign and forced grasping of the right hand. No sensory loss could be detected over the body. On Oct. 17, 1935, a ventriculogram was made by inserting a needle through the defect in the left parietal region. This demonstrated an evagination of the left lateral ventricle toward the parietal defect, the ventricular wall extending to within 1 cm. of the surface of the brain. The right lateral, third and fourth ventricles were also markedly dilated. No air was in the posterior cistern but some globules of lipiodol still remained in this region. It seemed that there must be an obstruction in the posterior fossa of the skull, so on Oct. 21, 1935, a suboccipital operation was undertaken under avertin anesthesia supplemented with ether. The left lateral ventricle was punctured early and fluid in abundance escaped. The operation progressed uneventfully until the cerebellar hemispheres were exposed. Suddenly the cerebellum began to herniate through the wound. Puncture of the cerebellar hemispheres revealed no hematoma; the needle in the lateral ventricle was draining well. It was impossible to explore around the sides of the cerebellum. No explanation of the sudden pressure could be found. It was impossible to proceed with the exploration. The wound was closed over the tense cerebellum. The patient naturally was much distressed, developed respiratory difficulties and died on Oct. 28, 1935.

Postmortem Examination

Autopsy was performed 12 hours postmortem. Apart from the nervous system there was found an acute purulent bronchitis and early bronchopneumonia, acute distention of the right side of the heart, endometrial polyp and a menstruating uterus. The dura mater was adherent to the brain in the left parietal region. The posterior surface of the cerebellum was badly lacerated. There was a marked internal hydrocephalus, especially of the left lateral ventricle, as noted in the ventriculogram. A small nodule of tumor 4 mm. in diameter was found on the inner surface of the dura mater just back of the right frontal sinus. A small warty tumor was present on each third nerve just at its entry into the dura

mater. A larger similar tumor was attached to the dura mater back of the posterior clinoid process where the left fifth nerve penetrated this membrane. A tumor 5 mm. in diameter lay in the left internal acoustic meatus and another 1 cm. in diameter in the right acoustic meatus. A similar tumor 5 mm. in diameter lay in each jugular foramen. The sixth, fourth and twelfth nerves were not accompanied by tumors. Two other nodules lay on the dura mater of the suboccipital fossa at some distance from the foramens. Several small nodules of tumor of varying sizes were found on the falx cerebri and tentorium cerebelli. Opposite the bodies of the first and second cervical vertebrae was a mass of tumor 3 by 5 by 2 cm. in diameter which had dislocated the spinal cord backward and to the left but had not compressed it. It was attached by a broad base to the dura mater anteriorly. At the level of the body of the sixth cervical vertebra lay another tumor 1 cm. in diameter on the posterolateral aspect of the spinal cord attached to, but not arising within, the spinal root. There were numerous nodules of tumor a few millimeters in diameter on the roots of the cauda equina. The leptomeninx was greatly thickened throughout the length of the spinal cord. Many of the dorsal roots were swollen slightly in fusiform fashion but no tumors of any size of the spinal roots were found. Many of the roots of the cauda equina looked like strings of small beads. One small nodule was found on a motor root in the upper lumbar region.

Microscopic Examination

The left oculomotor nerve was enlarged to 4 mm. in diameter. At its largest diameter a transverse section showed by microscopic examination that most of the nerve fibers were pushed to one side but others were scattered around most of the periphery. Occasional bundles and isolated fibers were seen deep within the cross section. The greater part of the cross section was occupied by a tumorous proliferation of spindle shaped elongated cells with scanty cytoplasm and elongated nuclei containing abundant chromatin. These cells formed broad bands interlacing in every direction. The nuclei were irregularly distributed with little or no tendency to lie in rows. In some areas the cells formed whorls. In many of the whorls one could identify an axis cylinder (Fig. 27) sometimes with a myelin sheath (Fig. 34). In most of the larger

whorls no nerve fibers could be identified, but often a large cell with clear cytoplasm and a vesicular nucleus looking like a Buengner cord could be seen (Fig. 33). In the non-tumorous areas the axis cylinders, myelin sheaths and endoneurium were easily recognized. The endoneurium was often thickened and hyalinized. But it was very difficult to recognize anything that might have been Schwann cells. Rarely one could find what appeared to be a sheath distinct from the endoneurium. In the whorls it was often possible to distinguish two portions — an outer, denser ring staining heavily with eosin, and an inner proliferation, semisyncytial in character and with more delicate protoplasm, staining less densely with eosin (Fig. 38). The outer dense ring was continuous with the endoneurium, the inner cells were of a more delicate cytoplasm. The cells of the tumor where they ran in bands resembled closely those of the endoneurium of the uninvolved portion of the nerve. There was no definite boundary between tumor and uninvolved nerve. The cells of the tumor formed abundant fibrils of reticulin. So did the cells in the outer layers of the whorls. The inner portions of the whorls were often entirely free from reticulin (Fig. 28).

The right third nerve measured 5 mm. in diameter at its widest point. It was more completely transformed into tumor but numerous nerve fibers persisted around the entire periphery. Otherwise conditions were very similar to those in the left third nerve and no special description seems necessary.

The grand sympathetic trunk appeared microscopically to be quite normal. The right ilioinguinal nerve appeared normal microscopically. The lower cord of the left brachial plexus was normal microscopically. The upper cord of the lumbosacral plexus was normal microscopically except for one funiculus which contained in its center a tumorous proliferation. The perineurium of this funiculus was greatly proliferated, there being a dense outer portion resembling a normal perineurium and then a proliferation of loose fibrous tissue five to ten times as great in diameter as the normal perineurium. Then followed a layer of apparently normal nerve fibers, within which was the tumorous proliferation that occupied more than one-half the cross section of the funiculus. The affected funiculus was enlarged to twice the diameter of the next largest funiculus in the nerve trunk. Within the tumor one saw

clearly two types of tissue — elongated cells in great whorls and bands, and a reticulated, apparently syncytial tissue. The latter tissue resembled closely the Remak bands of the uninvolved portion of the nerve; the former the endoneurial cells. Often within one of the large whorls would be found a small amount of the reticulated tissue enclosing a myelin sheath or naked nerve fiber. The tumorous mass was fairly sharply outlined but there was no capsule and many nerve fibers and myelin sheaths persisted within the growth.

There appeared to be no diminution of the myelinated nerve fibers in the optic nerves peripheral to the chiasm.

One of the flat nodules in the falx cerebri was sectioned. It proved to be a dense, disc-like mass of collagenic tissue, containing very few nuclei, lying between the two layers of the dura mater. Another larger one was a typical psammomatous meningioma.

Sections at various levels along the spinal cord demonstrated that the leptomeninges were greatly thickened along the entire spinal cord. Occasionally small tumors were found in the dorsal roots, rarely on the motor roots. These tumors looked sometimes like typical neurinomas, and sometimes were composed of whorls of cells similar to a meningioma. In addition rarely a root would be seen with a different appearance; in these roots the nerve fibers and myelin sheaths had almost entirely disappeared and their places were filled by a trabeculated, lightly stained cytoplasm (Fig. 32). A small amount of fibrous tissue subdivided this trabeculated material into cords. The appearance was very similar to that of the neuroma forming on a transected peripheral nerve.

The tumor of the right vagus nerve measured 1 cm. in diameter. It looked microscopically, for the most part, like a typical neurinoma. But in some areas small whorls were formed so that the tissue here had the appearance of a meningioma, (leptomeningial endothelioma, meningothelioma). In some of these whorls axis cylinders could be identified (Fig. 29). The nerve fibers lay around the periphery or penetrated in bands of considerable number into the growth. Myriads of reticulin fibrils permeated the tumor everywhere.

The tumor of the left trigeminal root measured 12 mm. in diameter. It had a sort of hilum into which the nerve plunged to

break up into bands of fibers which passed through the tumor in all directions. The tumor tissue had all the appearance of a typical neurinoma. The cells lay in broad bands and formed myriads of delicate reticulin fibrils.

The right acoustic tumor measured 16 mm. in diameter, the left 12 mm. The right tumor was a typical acoustic neurinoma for the most part but in small areas whorls were formed (Fig. 37). The left acoustic tumor was very similar (Figs. 35 and 36).

The tumor of the left vagus nerve in the intracranial cavity measured 6 mm. in diameter. It had evidently arisen within the nerve because no nerve trunk was found on the surface of the growth, but myelinated fibers were scattered in the peripheral layers of the tumor. The tumor was an inextricable mixture of the two types of tissue we have been describing, consisting of broad bands of elongated delicate cells with elongated nuclei tending to lie in rows, separating masses of other cells forming small whorls. The whorls were not cross sections of the elongated cellular bands. Such cross sections were also found and were readily distinguished from the whorls. Many of the whorls originally formed around nerve fibers, since in some instances such whorls with myelin sheaths in the center were found in the periphery of this tumor.

The spinal cord was surrounded by a thickened and densely collagenic pia-arachnoid membrane within which the nerve roots were often widely dispersed. Around these roots could be found arachnoidal clusters and within them neurinomatous nodules. The fourth lumbar dorsal nerve root on one side contained a typical neurinoma about 1 mm. in diameter (Fig. 39 (N)). The delicate, greatly elongated cells of this tumor ran in long bands; the nuclei showed a definite tendency to align themselves in rows and the cells were forming numerous reticulin fibrils. In the opposite posterior root was a similar neurinomatous formation of larger size which extended into the posterior horn of the spinal cord (Fig. 39). The reticulin fibrils of the tumor followed into the spinal cord almost to the central canal in great numbers. The intramedullary tumor reproduced the true neurinomatous tissue and was not what is ordinarily called a "neurinoma centrale" in which no reticulin is produced.

In addition there was a small tumor of entirely different appearance in one funiculus. This small tumor was composed exclusively

of small tight whorls of cells, giving it the appearance of a meningothelioma (Fig. 40). It was, however, entirely within the nerve root and, although it reached the perineurium, did not appear to have arisen from this structure which was clearly distinct. Although the tumor was fairly sharply circumscribed, it had no definite capsule and within it were nerve fibers scattered about both within and without the whorls.

Moreover, the posterior raphe of the spinal cord was open in places almost down to the central canal. The tumor at the first cervical level, which had been partially removed at operation, contained considerable scar tissue and hemorrhage. It was composed of strands of dense cytoplasm seeming to anastomose like those of cardiac muscle, the strands being separated after the manner of an accordeon. In this meshwork of cytoplasmic strands the numerous nuclei were embedded with no tendency to palisading. Blood vessels were rare. The neoplastic cells did not form reticulin or collagen. The spinal cord had been pushed to one side, compressed and gliosed at this level.

The tumor at the sixth cervical level was firmly attached to the dura mater over a broad base. It resembled in many parts the tumor at the first cervical region, but in addition it contained numerous whorls of cells often hyalinized and occasionally calcified (Fig. 30). In some areas these whorls were so numerous and compact as to resemble closely the similar formations in the nerve roots.

The meningeal tumor over the frontal dura mater resembled so closely in certain areas an acoustic neurinoma that it could with difficulty be distinguished.

The external surface of the brain did not appear abnormal except over the parietal herniation. Otherwise the convolutions appeared normal. Nor did the ventricular surfaces appear abnormal except underneath the parietal hernia. There were no nodules or abnormal irregularities of the ventricular surfaces. On cross section nothing abnormal could be seen with the naked eye, but microscopic studies revealed very extensive abnormalities of the finer structure of the cerebral hemispheres and basal ganglia. Nothing abnormal was found in the cerebellum or bulb, but scattered throughout the cerebral cortex and subcortical regions were collections of abnormal cells a few millimeters in diameter. In the

subcortex they appeared clearly to be large distorted neuroglial cells with vascular processes extending to vessel walls. But in the cortex the nature of these cells was not so clear. They had nuclei which were most often vesicular, like those of neurons, but varied greatly in size. The cells gave off numerous processes similar to neuroglial cells and these processes were not impregnated by Bielschowsky's method nor by the gold sublimate method of Cajal, at least in Globus' modification. They were impregnated by the silver carbonate method of Hortéga if the impregnation were pushed beyond the limit of specificity. The cells contained no Nissl bodies. These cells seemed clearly to be of the same nature as the abnormal cells so often described in tuberous sclerosis. In addition the cyto-architectonics of the cortex were grossly deranged in the neighborhood of these abnormal accumulations of cells. Displaced and abnormally oriented nerve cells were often found in the deeper layers of the cortex or even in the subcortex.

The nodules on the roots of the cauda equina were microscopically typical neurinomas with nerve fibers penetrating for a short distance only in the peripheral portions. Although rather sharply circumscribed in some areas, the tumor cells penetrated diffusely between the nerve fibers for 1 or 2 mm. beyond the apparent margin.

Discussion

Such cases of multiple tumors of the nature of those we have just described are not excessively rare and have been recorded by Agostini,[1] Schnyder,[45] Bielschowsky and Rose,[6] Penfield and Young,[38] Orzechowski and Nowicki,[36] van Bogaert,[57] Foerster and Gagel,[16] Struwe and Steuer,[54] Schairer [43] and many others. The association of these various pathological alterations is therefore due to more than chance. There must be some underlying cause for their frequent association in the same patient. This cause has been sought in a common embryological origin for the affected structures. In order to evaluate this hypothesis, it is necessary to make a detour into the structure and development of the nervous system.

Anatomy: It is not needful here to enter into controversial matters or to review the history of the development of our present views concerning the structure of nerves. These matters can be

found discussed in detail in the masterly studies of Nageotte.[34] Suffice it to say that we must recognize in the nerve trunks three categories of structures: (1) the nerve cells, axis cylinders and myelin sheaths; (2) the capsular cells of the ganglia, the Schwann cells, and Remak cells; and (3) the endoneurial-perineurial connective tissue. The endoneurium and perineurium have essentially identical cellular composition, being composed largely of fibroblasts which form interstitial fibrils of reticulin, collagen, and rarely of elastin, together with a variable number of other cells of connective tissue origin. Those collagenic fibrils of the endoneurium which run longitudinally in the intervals between the nerve fibers are known as the fibers of Key-Retzius. The entire endoneurial-perineurial system constitutes the sheath of Henle if we read that ancient author correctly. But more important for our present study is another structure which we will follow Nageotte in calling the Plenk-Laidlaw sheath, since these authors (Laidlaw [26]) have so clearly demonstrated it. This structure is a delicate network of argyrophilic fibrils which closely invests the medullated nerve fibers and may even extend with them for a short distance into the spinal cord. We will return to this sheath later. The myelin sheath is doubtless a part of the axis cylinder, since it is formed also around such nerve fibers in the central nervous system, but Speidel [48] has recently demonstrated clearly that its formation is precipitated by the proximity of cells of the second category in the peripheral nerves. These latter cells migrate out along the developing nerve fibers and apply themselves to the surfaces to form sheaths about the fibers which have long been known as the Schwann cells of the myelinated fibers and the Remak cells of the unmyelinated fibers. These cells are believed by Nageotte to form a syncytium. If so, Speidel's studies indicate that the syncytium is a secondary formation. It matters little for our present purposes. More important is the fact that on the outer surface of the Schwann cells is a condensation called the neurilemmal sheath or membrane of Schwann.[46] Such a condensation about the Remak cells is less evident. It should be remarked here that, although the neurilemmal sheath is easily seen in adult normal nerves, it is extraordinarily difficult to demonstrate clearly the cytoplasm of the Schwann and Remak cells. Moreover, there is some doubt concerning the relation of the membrane of Schwann

to the Plenk-Laidlaw sheath. Masson [29] is inclined for various reasons given in his papers to believe that the Plenk-Laidlaw sheath is an argyrophilic network within the membrane of Schwann and coterminous with it. This would make it a sort of basement membrane around the Schwann cell, and quite a nuisance for the present study since it is best demonstrated by methods that also demonstrate sharply the interstitial substances formed by the fibroblasts of the endoneurium. It is quite obvious, in the absence of any utilizable method of differentially emphasizing the cytoplasm of the Schwann and Remak cells, that the problem of differentiating the origin of tumor cells from Schwann cells or endoneurial cells should be particularly aggravated by the fact that they both form interstitial substances reacting in identical fashion to staining methods. This fact is essentially the crux of the controversy between the two schools of thought represented at the present time by Penfield [37] and Masson.[29]

Embryology: We have above remarked the tendency to explain the association of multiple disseminated tumors involving the nervous system by assuming that the structures in which the neoplastic transformation occurs have a common embryological source. It may be well at this point to enumerate the most important types of pathological alterations concerned. They include such apparently disparate lesions as tuberous sclerosis of the brain, glioma of the optic chiasm, melanoblastosis of the leptomeninx, leptomeningotheliomas, neurinomas of the acoustic nerve, neurinomas of the peripheral nerves and their roots, interstitial hypertrophic neuritis, disseminated neurofibromatosis, cutaneous nevi, cutaneous hyperpigmentation, subcutaneous elephantiasis, and many others. All of these pathological formations have been related to normal structures associated with the nervous system by various authors, the hyperpigmentation to the cells of Langerhans (Masson [32]), the nevi to Wagner-Meissner corpuscles (Masson [30]), the disseminated neurofibromatosis to the endoperineurium (Penfield and Young [38]), the interstitial hypertrophic neuritis to the Schwann cells (Boveri [8]), the neurinomas to the Schwann cells (Verocay [58]), the leptomeningotheliomas to the arachnoidal granulations (Schmidt), the melanoblastomatosis to the leptomeningeal melanoblasts, while the association of glioma of the optic chiasm with the other lesions was pointed out long ago by Emanuel and

the relation of the cerebral lesions of tuberous sclerosis to those found in disseminated neurofibromatosis by Bielschowsky and Rose.[6] How far can these normal structures involved be said to have a commmon embryological origin? In the beginning we must put some limit to our search. It is quite clear that all have arisen by progressive differentiation from the same ovum. It helps us little to go back even to the primary germ layers. The doctrine of the specificity of the germ layers is no longer tenable and it is generally accepted by embryologists that more than one primary germ layer can contribute to a normal structure of homogeneous histological structure when fully developed (Stone [50]). With minor exceptions the nervous system, both central and peripheral, develops from the ectoderm along the medullary groove, and here we must distinguish early the medullary tube and the neural crest. The neural crest is a group of cells that lie in the angle between the medullary tube and the surface ectoderm. It has been shown by embryologists that cells from this formation migrate outward to form the dorsal roots of the spinal nerves, the dorsal root ganglia, the sympathetic nervous system, the medulla of the adrenal gland (Kohn [24]) and other chromaffin structures. The dorsal root fibers are joined also by the prolongations of cells in the anterior horns of the spinal cord to form the spinal nerves (Held, Cajal). And it was further demonstrated by embryological experimentation, following the lead of Harrison [21] that, if the neural crest be removed in amphibian larvae, not only is there no formation of dorsal spinal roots and sympathetic nervous system, but also that motor fibers of the anterior roots grow out without any accompanying Schwann cells. It is generally accepted, therefore, that the Schwann cells (also the Remak cells) are of neural crest origin with minor exceptions (olfactory nerves, Disse). The same cannot be said of many other structures covering the nervous system. The sheath cells of the Wagner-Meissner corpuscles cannot be proved in the same manner to arise from the neural crest since the lower vertebrate forms used for such experimentation do not have these corpuscles. They are believed, however, on embryological grounds to be analogous to Schwann cells (Klein [23]). The cells of Langerhans also have not been proved to have such an origin, although the experiments of Dushane [15] indicate that such may be the case in amphibia. The endoneurial-perineurial

tissue has been generally considered to arise *in situ* by condensation of the mesenchyme along the outgrowing nerves. An attempt was made by Harvey, Burr and von Campenhaut [22] to prove that the leptomeninx, stated by Tarlov [55] to be continuous with the endoperineurium, arises from the neural crest, an idea previously expressed by Oberling [35] and supported by the presence in it of melanoblasts. And these attempts to prove an origin from the neural crest for the leptomeninges have found support (Raven [41]), yet no similar demonstration has been made for the endoperineurium. It is evident that the suppositions underlying the attempts to explain the association of these multiple tumors on the theory of a common embryological origin are not thoroughly substantiated. In particular the endoneurium-perineurium seems very unlikely to be of neural crest origin which makes all the more damaging the contention of certain authors (Penfield) that neurinomas arise from the perineurium. It is for this reason that Masson defends so vigorously the schwannian nature and origin of the neurinomas.

Pathology: The pathological arguments for the nervous nature of these lesions are largely by analogy. In the lesions of von Recklinghausen's disease structures are found that resemble Wagner-Meissner corpuscles (Brögli,[10] Masson); the cell clusters of nevi are supposed to be an attempt to form the same structures (Masson [31]); also the palisading of the nuclei in neurinomas is interpreted as organoid production comparable to the supporting apparatus of the tactile corpuscles (Masson [29]). On the other hand, Penfield [37] points out analogies of the structure of neurinomas to the perineurial tissues. He remarks that among the tumor cells are many fibrils that react like collagen and reticulin and concludes therefore that the cells must be fibroblastic. His assumption is that only fibroblasts can form reticulin and collagen, an assumption denied by Masson and unsupported by general histological data unless one gives a very wide interpretation to the term fibroblast, since it is generally recognized that many endothelial cells form argyrophilic substances that react to silver in a manner similar to reticulin. The fact that in the cells of these tumors delicate fibrils are found that stain blue with phosphotungstic acid hematoxylin and by methods that stain neuroglial fibrils has been used both as an argument for the fibroblastic nature

of the neurinomas (Penfield, Mallory) and for their neuroglial nature (Lhermitte). It is obviously an inconclusive argument since fibrils with the same staining reactions can be found in cells both of mesodermal and of neuro-epithelial origin, and in many other epithelial cells as well.

We will turn now to our own observations on pathological material. A comparison of our descriptions and microphotographs in the 2nd case (E.N.) seems to us conclusive that we are there dealing with the same alterations that have been so often described in cases of so-called hypertrophic interstitial neuritis (Wolf, Rubinowitz and Burchell [61]). The whorls of cells about the axis cylinders have been interpreted by Boveri,[8] Bielschowsky,[5] Marie and Bertrand,[27] Souques and Bertrand,[47] Roussy and Cornil [42] and others as schwannian proliferations. We feel that such an interpretation is unjustified in our case. We saw occasionally in the center of a whorl a distinct cell with abundant light cytoplasm, which stained yellow with van Gieson's and red by Masson's trichrome stain, and a vesicular nucleus (Fig. 33). Rarely a larger mass of apparently syncytial structure containing several nuclei with delicate cytoplasm and no reticulin was found (Fig. 38). These formations resembled exactly those described and illustrated by Marie and Bertrand.[27] But such formations had to be searched out with difficulty. There was no doubt that many of the whorls formed around myelinated nerve fibers, perhaps all of them, but we could see no good reason to suppose that they arose from Schwann cells. On the contrary, they seemed clearly to be continuous with and identical in structure with the endoneurium and perineurium. They were composed of elongated spindle shaped cells with dense scanty cytoplasm and wrinkled crenelated nuclei, interspersed by fibrils of collagen and reticulin. We can see no good reason to suppose that these whorls were not formed by the endoneurial tissue. If the formations in nevi and again in other peripheral tumors can be conceived to resemble Wagner-Meissner corpuscles, whose supporting cells are accepted to be of schwannian origin (Klein [23]), these whorls as clearly resemble the corpuscles of Vater-Pacini which contain only a central column of schwannian cells and a laminated capsule of mesodermal cells.

It is furthermore remarkable that in many tumors the areas of whorls passed over by gradual transition into other areas with the

typical classical structure of neurinomas. If the whorls are of endoneurial origin, we can find no good reason to suppose that the neurinomatous formations are not also. In his classical study of experimental and spontaneous schwannomas Masson [29] states that "in certain examples of generalized neurofibromatosis, studying nerves of apparently normal dimensions, several authors (Verocay, Pick and Bielschowsky) have seen microscopic tumors composed of Schwann cells in proliferation." We have perused carefully the studies which Nageotte and Masson have made of schwannian proliferations and we have repeated and confirmed their experiments; we have studied known schwannian proliferations in human amputation stumps; we have carefully studied the published observations of Verocay,[58] Pick and Bielschowsky,[39] and many others; we have studied numerous small neurinomas from both of our cases, and we have never been able to convince ourselves that any one of them arose from schwannian structures. They can be seen to arise within nervous funiculi, with no attachment to the perineurium, but whether from the Schwann sheaths or endoneurium we were never able to determine. We were, however, in the 1st case (B.S.) able to find several nodules in the spinal nerve roots of definitely neurinomatous structure arising unmistakably from the perineurium.

We were much more impressed by the resemblance of the tumors in our 2nd case (E.N.) to the ordinary leptomeningioma (Figs. 40, 29, 37). If we make abstraction of the nerve fibers which were contained in parts of them we fail to find any other differences. Also one of the leptomeningial tumors in this case had a structure identical with that of the acoustic tumor. The more we have studied the tumors from this remarkable case the more we have been impressed with the close relation between the neurinomas and leptomeningiomas; and the conviction has grown upon us that the Schwann cells, although they may undergo a slight hypertrophy secondary to the degeneration of nerve fibers in the tumor, have nothing to do with the tumor formation. This experience has shaken our faith in the schwannian origin of any case of hypertrophic interstitial neuritis. We see in the formations depicted by Roussy and Cornil [42] a proliferation of the Key-Retzius apparatus and, although in the case reported by Marie and Bertrand we can believe that there was some hypertrophy of the

schwannian apparatus such as we have also seen (Fig. 38), we believe such schwannian proliferation plays a purely secondary rôle.

In the study of our 1st case (B.S.), which is unquestionably one of generalized neurofibromatosis, we have found, and described above, typical schwannian hypertrophies secondary to the degeneration of nerve fibers, but rarely anything resembling tumorous proliferation (Figs. 5 and 6). After careful scrutiny of dozens of sections we could never find budding or branching of nerve fibers, which instead always ran in parallel bundles (Fig. 10), the only changes in them being degenerative. These bundles seemed to us to be entirely similar to those described by Bielschowsky [5] in Boveri's case. The changes we observed in both the schwannian and Remak apparatus were essentially reactive or degenerative, rarely proliferative. On the other hand, the vast overgrowth of the nerves of this case seemed to us to be clearly of fibroblastic nature and we see no reason to consider it to be derived from any other tissue than the endoperineurial sheaths. This fibroblastic proliferation underwent a widespread liquefaction necrosis which by the accumulation of fluid dispersed the remaining cells widely. It is interesting to note the elongated bipolar cells of this tissue (Fig. 9), which were doubtless similar to those figured by Bruce and Dawson [11] in their case and interpreted by them as neurogenic cells which, forming in chains, were supposed to generate nerve fibers.

As a result of our observations we have become very skeptical of the schwannian theory of the origin and nature of any tumors of the peripheral nerves. Such publications as those of Stout,[51] Martin and Déchaume,[28] Geschickter,[20] Stewart and Copeland,[49] or of Foot,[18] advance the solution of this particular problem not at all. The schwannian theory seems to us to be more likely to explain satisfactorily the origin of such tumors as those described by Cohn,[13] and by Stout,[52] also Bergstrand,[4] in the peripheral nerves, by Masson [29] in the palate, or by Cid [12] in the scalp; but when extended to the ordinary neurinomas we find the evidence unconvincing and it seems to us unlikely to become more convincing until better and more specific technical methods are available. The neural crest theory seems as little likely to prove fruitful. If we accept an origin from the neural crest for the leptomeninx the findings in our 2nd case (E.N.) indicate that it must be ex-

tended also to include the endoperineurium. There is to our knowledge no justification for such an extension.

But if we reject the schwannian hypothesis many questions remain to be answered. What of the association of all these various pathological alterations? What of the undoubted fact that tumors of the nerves have a distinctive structure not found in fibroblastomas elsewhere? What of the lesions in the brain? Many of the latter are composed of elongated cells with sausage shaped nuclei bearing such a close resemblance to malignant tumors of the peripheral nerves that they have been called a lemmoblastosis (von Sántha [60]). Antoni [2] suggests that part of the neural crest tissue in such cases has been incorporated in the brain. Certainly if those cases that have tumors or malformations affecting both the neuraxis and peripheral nerves are to be explained on the basis of a maldevelopment, then the fault must involve both the medullary plate and the neural crest. And in such a case what is to prevent maldevelopment occurring in associated structures even if they are not derived embryologically from the neurectoderm? Speidel [48] has shown that the proximity of a Schwann cell is necessary for the production of a myelin sheath by a peripheral axis cylinder, but that this stimulus alone is not sufficient; many of them remain unmyelinated even in the presence of a Schwann (Remak) cell if they do not have the proper central connections. If the neurectodermal structures are malformed, associated mesodermal structures, lacking the proper stimulus, will also not develop properly, and thus possibly also be liable to neoplastic change. The whole development of inductive embryology by Spemann and his school indicates that the orderly development of the body is due to a concatenation of mutually interacting forces, the derangement of any one of which will deviate the orderly unfolding of the rest. So the association of tumors of various structures may be explained on another basis than that of a common embryological derivation, and we see no reason why in generalized neurofibromatosis tumors and malformations should not simultaneously occur in structures of both neurectodermal and mesodermal derivation. This attitude toward the problem has already been suggested by Worster-Drought, Dickson and McMenemey,[62] and seems to us very reasonable. As for the distinctive structure of tumors of the peripheral nerves, certainly true in spite of the attempts of some pathologists

(Krumbein [25]) to belittle it, it does not necessarily argue in favor of a neurectodermal origin of the tumor cells but possibly only for a small degree of specialization from the fibroblasts of other connective tissues, just as certain cells of the general matrix of the leptomeninx, although derived from the general mesenchyme, have sufficiently specialized to form tumors with a distinctive structure.

Summary and Conclusions

1. Two cases of multiple tumorous proliferation of nervous and associated tissues have been described.

2. The cells of Schwann are believed to play a minor and secondary rôle in the production of the tumors of the peripheral nerves in these cases.

3. The origin of tumors of the peripheral nerves remains for us doubtful because the cells of the two possible sources (Schwann cells and endoneurium) form similar intercellular substances and specific staining or impregnation methods for identifying their cytoplasm have not been devised.

4. Although we began our study thoroughly impressed by the attractive schwannian hypothesis, we have been persuaded by our own observations that a schwannian origin of the common neurinomas and spindle celled malignant tumors of the peripheral nerves is very doubtful. The admission of this doubt leaves intact our admiration for Masson's brilliant demonstration of the neurectodermal origin of nevi and leaves us still able to accept as probable the neurectodermal origin of such tumors as that described in the palate by Masson and Panneton, or in the scalp by Cid, or even many others of unusual structure in the peripheral nerves (Brandes,[9] Stout,[51] Cohn,[13] and Bergstrand [4]).

5. Because of the distinctive structure of the common circumscribed tumors of the peripheral nerves we do not favor the term *perineurial fibroblastoma* but believe it better, if the time honored *neurinoma* will not do, to use for them a more distinctive term such as *neurilemoma*, as proposed by Stout.[52]

Note: We wish to thank Dr. Joseph Brenneman, who referred the 1st case, Dr. Walter S. Priest, who referred the 2nd, and Dr. Paul Cannon, who performed the autopsies and kindly permitted us to examine the material.

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Case 1, B.S. Dorsal spinal root at the eleventh thoracic segment. Hematoxylin-eosin stain. × 60.

FIG. 2. Case 1, B.S. Anterior spinal root at the eleventh thoracic segment. Hematoxylin-eosin stain. × 60.

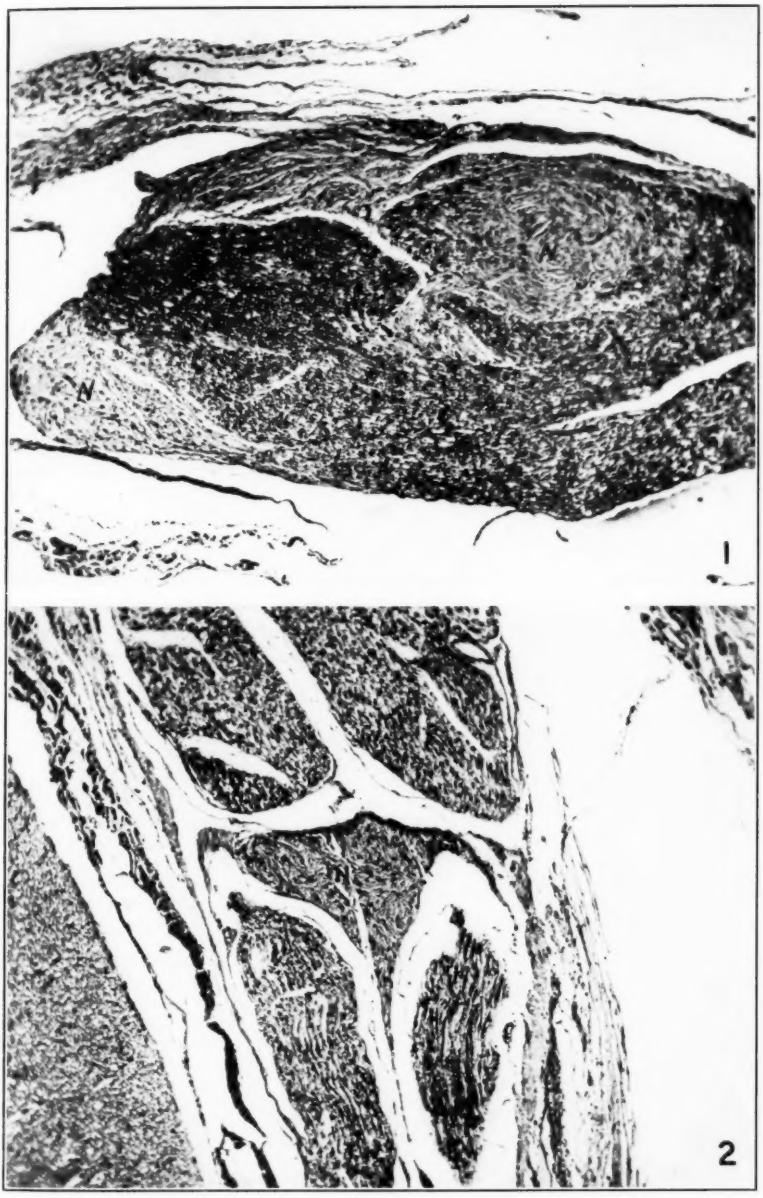


PLATE 2

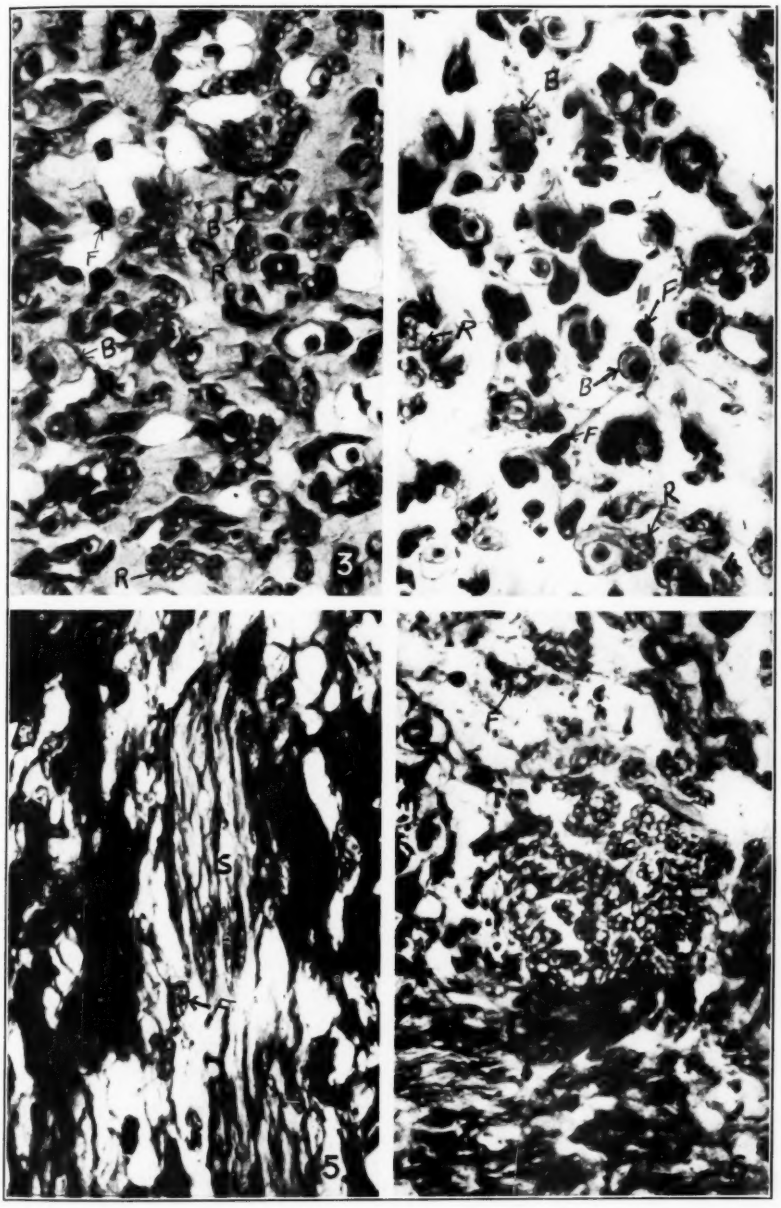
FIG. 3. Case 1, B.S. Lumbar plexus. Van Gieson's stain. × 700.

FIG. 4. Case 1, B.S. Lumbar plexus. Van Gieson's stain. × 700.

FIG. 5. Case 1, B.S. Lumbar plexus. Masson's trichrome stain. × 600.

FIG. 6. Case 1, B.S. Lumbar plexus. Masson's trichrome stain. × 600.

B = Buengner cords; F = fibroblasts; R = Remak bands; S = schwannian proliferations (in Fig. 5 in longitudinal section, in Fig. 6 in cross section).



Bailey and Herrmann Rôle of the Cells of Schwann

PLATE 3

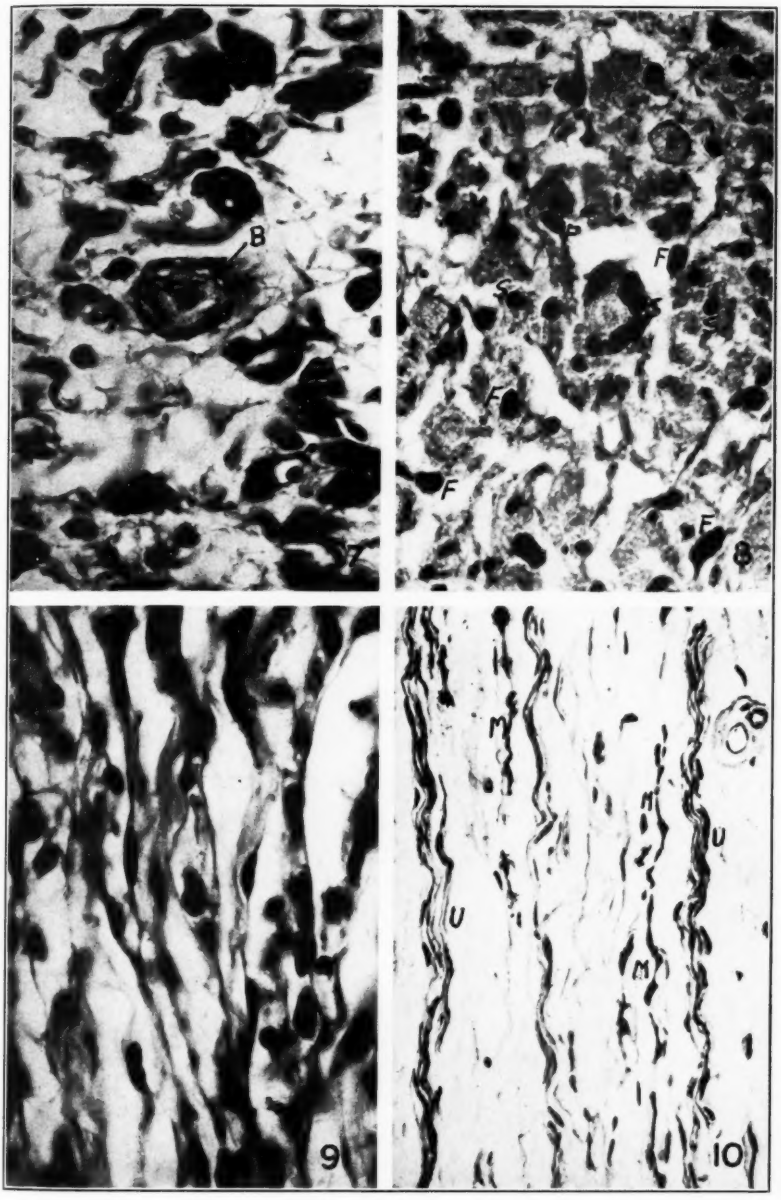
FIG. 7. Case 1, B.S. Tumor of the first cervical nerve. Masson's trichrome stain. × 600.

FIG. 8. Case 1, B.S. Lumbar plexus. Note the proliferation of schwannian nuclei about a myelinated nerve fiber. Hematoxylin-eosin stain. × 600.

FIG. 9. Case 1, B.S. Lumbar plexus, showing fibroblasts in a degenerated area. Hematoxylin-eosin stain. × 600.

FIG. 10. Case 1, B.S. Lumbar plexus. Bodian's method. × 300.

B = Buengner cord; F = fibroblast; M = myelinated nerve fiber; S = nuclei of Schwann cells; U = unmyelinated nerve fibers.



Bailey and Herrmann Rôle of the Cells of Schwann

Plate 4

Fig. 11. Case 1, B.S. Lumbar plexus, various stages of degeneration. Freeman's method. × 600.

Fig. 12. Case 1, B.S. Lumbar plexus, various stages of degeneration. Van Gieson's stain. × 600.

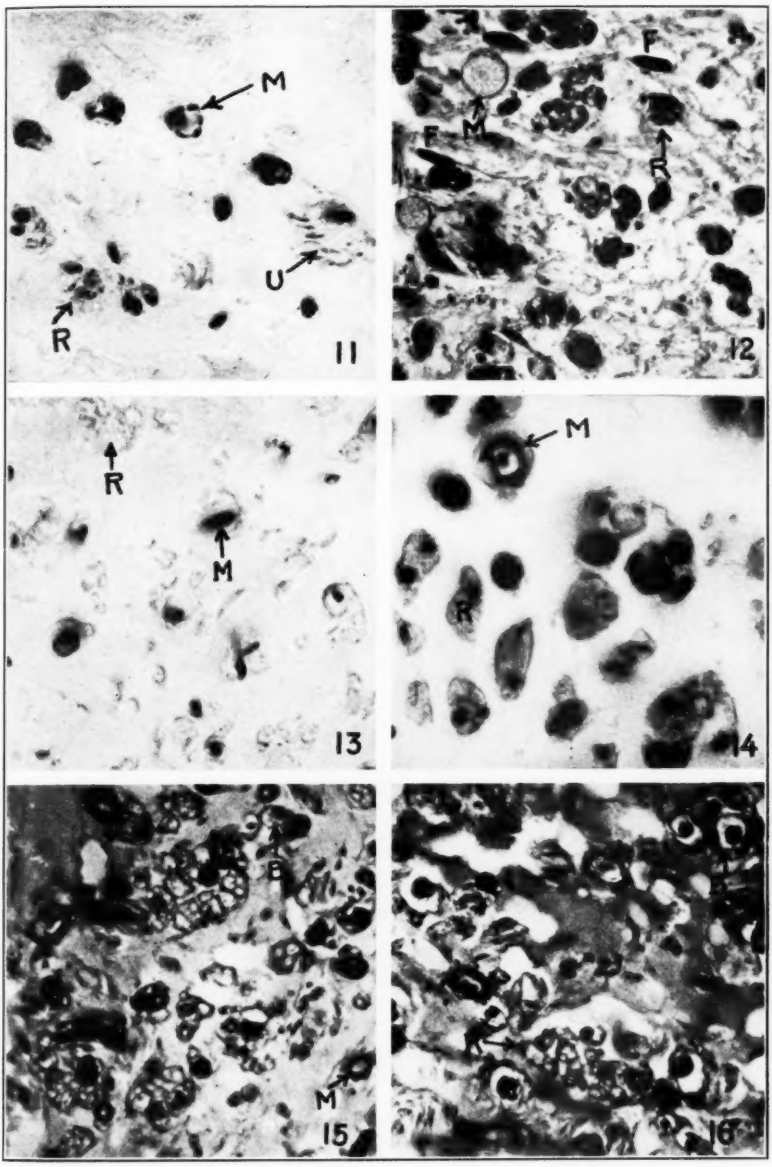
Fig. 13. Case 1, B.S. Lumbar plexus, various stages of degeneration. Freeman's method. × 800.

Fig. 14. Case 1, B.S. Lumbar plexus, various stages of degeneration. Weil's method. × 700.

Fig. 15. Case 1, B.S. Lumbar plexus, various stages of degeneration. Masson's trichrome stain. × 700.

Fig. 16. Case 1, B.S. Lumbar plexus, various stages of degeneration. Muci-carmine stain. × 700.

B = Buengner cord; F = fibroblasts; M = myelinated nerve fibers; R = Remak bands; U = unmyelinated nerve fibers.



Bailey and Herrmann Rôle of the Cells of Schwann

Plate 5

Fig. 17. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Freeman's method. × 800.

Fig. 18. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Weigert-Pal. × 700.

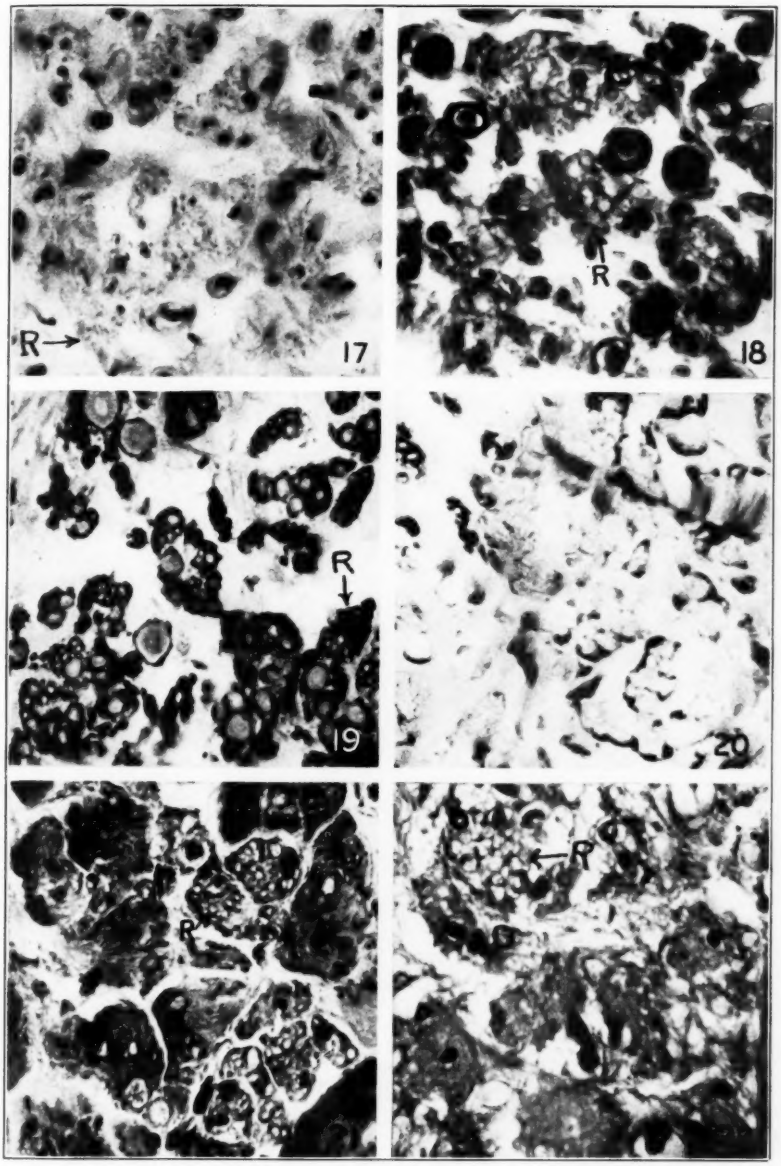
Fig. 19. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Foot's method. × 600.

Fig. 20. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Laidlaw's method. × 600.

Fig. 21. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Van Gieson's method. × 600.

Fig. 22. Case 1, B.S. Lumbar plexus, showing transformation of Remak bands into sclerotic masses. Masson's trichrome method. × 700.

R = Remak bands.



Bailey and Herrmann Rôle of the Cells of Schwann

PLATE 6

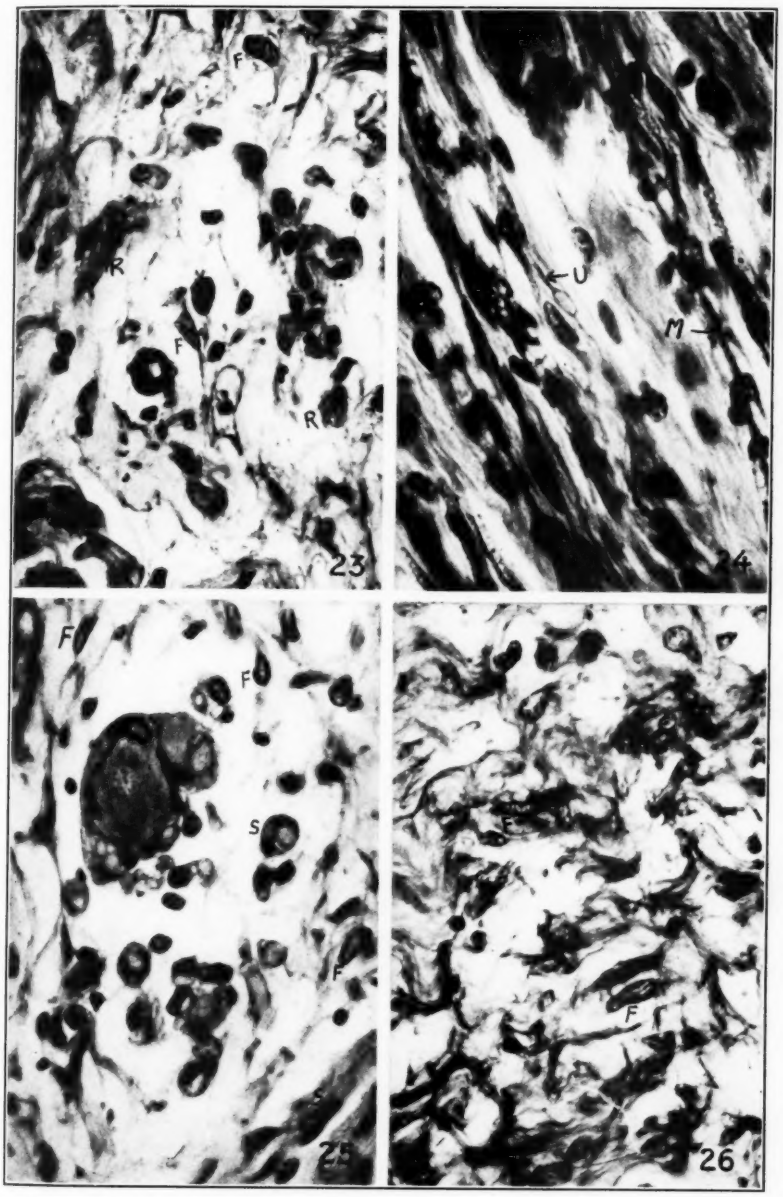
FIG. 23. Case 1, B.S. Sympathetic trunk. Hematoxylin-eosin stain. × 600.

FIG. 24. Case 1, B.S. Dorsal spinal root. Hematoxylin-eosin stain. × 600.

FIG. 25. Case 1, B.S. Sympathetic trunk. Hematoxylin-eosin stain. × 600.

FIG. 26. Case 1, B.S. Sympathetic trunk, from an area of pure fibroblastic proliferation. Hematoxylin-eosin stain. × 600.

F = fibroblasts; M = myelinated nerve fiber; S = Schwann cell; R = Remak band; U = unmyelinated nerve fiber.



Bailey and Herrmann

Rôle of the Cells of Schwann

Plate 7

Fig. 27. Case 2, E.N. Left oculomotor nerve. Hematoxylin-eosin stain. × 300.

Fig. 28. Case 2, E.N. Left oculomotor nerve. Perdrau's method. × 300.

Fig. 29. Case 2, E.N. Right vagus nerve. Hematoxylin-eosin stain. × 250.

Fig. 30. Case 2, E.N. Meningeal tumor at sixth cervical level. Hematoxylin-eosin stain. × 150.

A = axis cylinders.

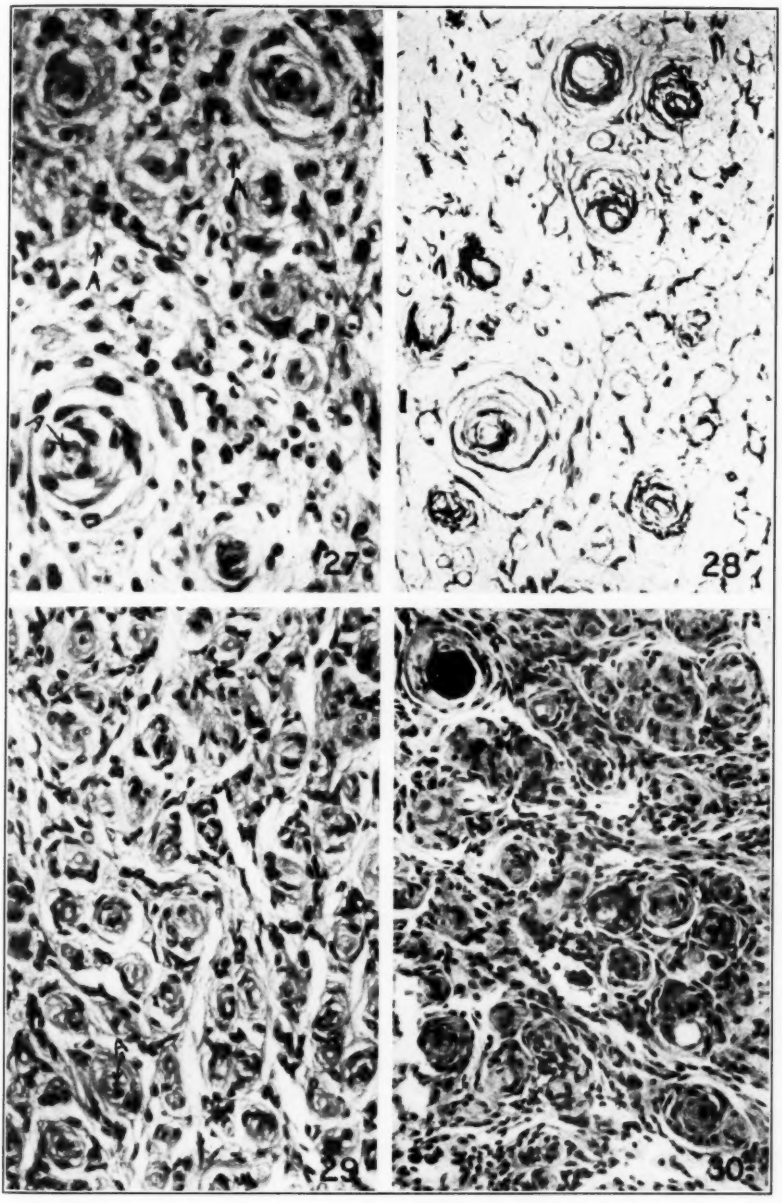


PLATE 8

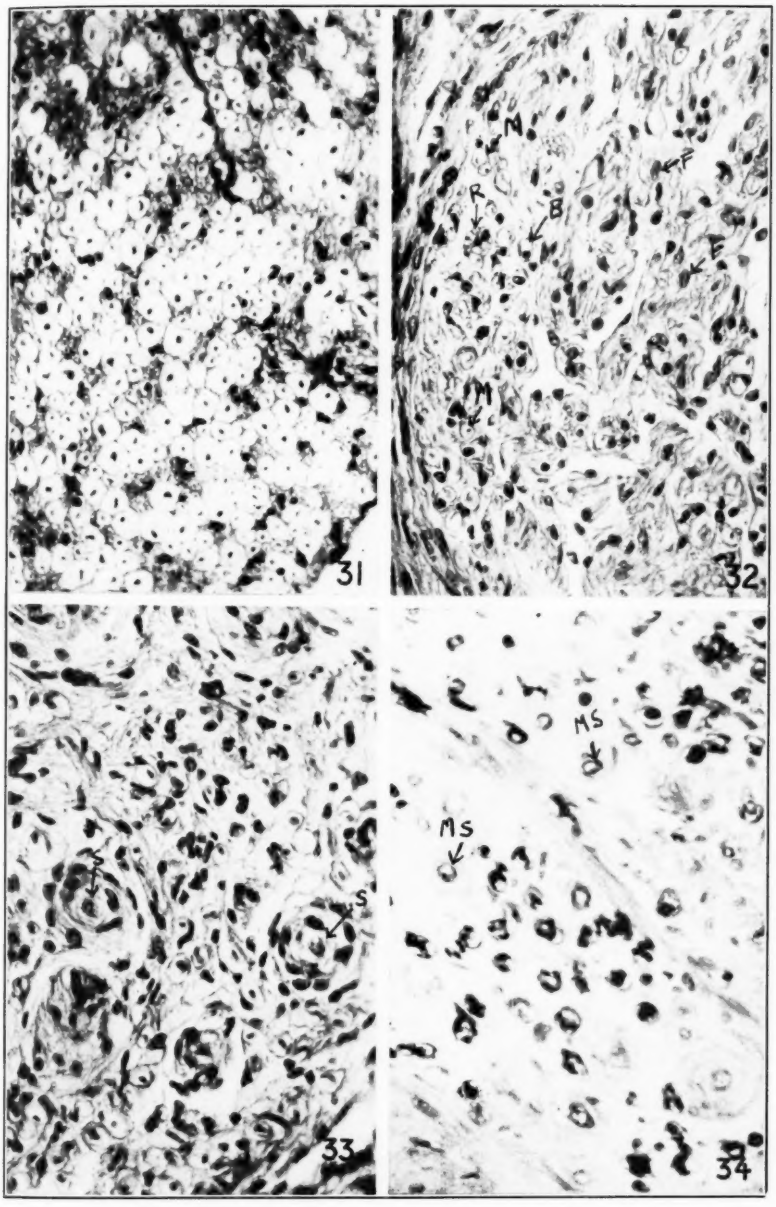
FIG. 31. Case 2, E.N. Normal funiculus from the dorsal root of the eleventh thoracic nerve. Hematoxylin-eosin stain. × 300.

FIG. 32. Case 2, E.N. Degenerated funiculus from the same nerve root. Hematoxylin-eosin stain. × 300.

FIG. 33. Case 2, E.N. Tumor of right oculomotor nerve. Hematoxylin-eosin stain. × 300.

FIG. 34. Case 2, E.N. Right oculomotor nerve. Loyez's method. × 300.

B = Buengner cord; F = fibroblasts; MS = persisting myelin sheaths within whorls; S = nuclei of Schwann cells within whorls from which axis cylinders have disappeared; R = Remak bands.



Bailey and Herrmann Rôle of the Cells of Schwann

Plate 9

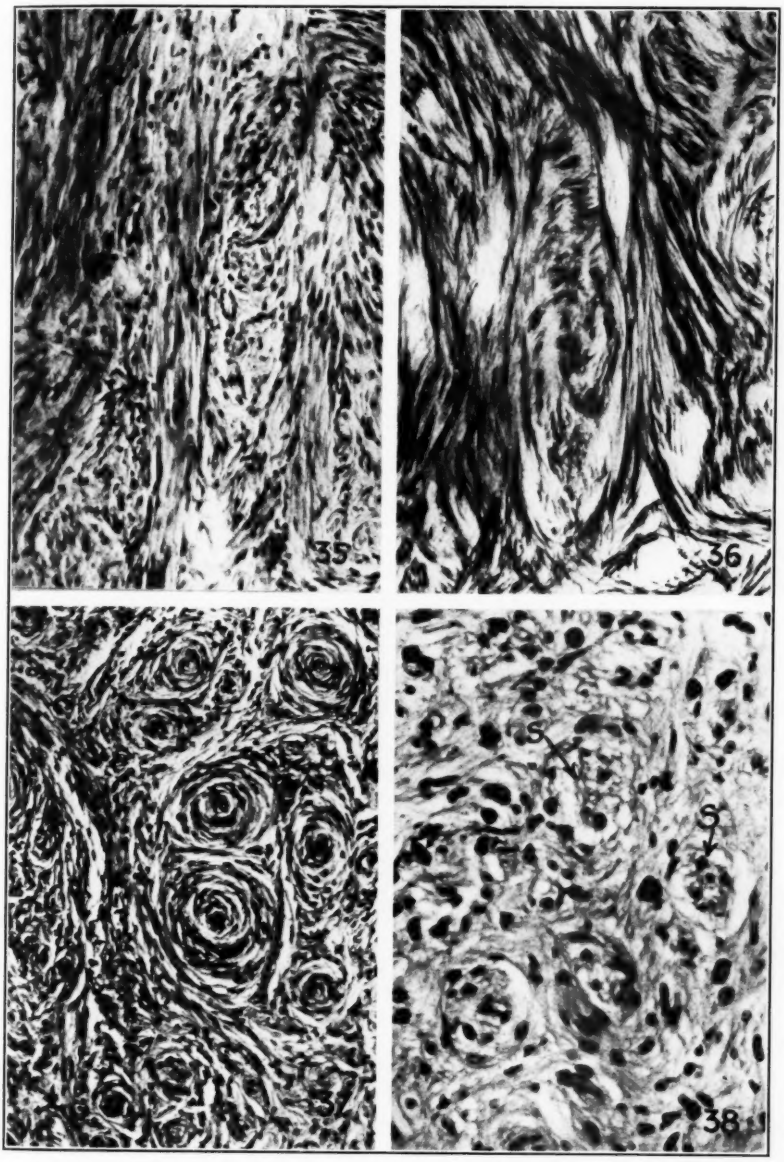
Fig. 35. Case 2, E.N. Left acoustic tumor. Hematoxylin-eosin stain. × 150.

Fig. 36. Case 2, E.N. Left acoustic tumor. Perdrau's method. × 150.

Fig. 37. Case 2, E.N. Right acoustic tumor. Hematoxylin-eosin stain. × 150.

Fig. 38. Case 2, E.N. Right oculomotor nerve. Hematoxylin-eosin stain. × 300.

S = schwannian proliferations.



Bailey and Herrmann Rôle of the Cells of Schwann

Plate 10

Fig. 39. Case 2, E.N. Cross section of the spinal cord at the fourth lumbar segment. Note the extension of the tumor of the posterior root into the left posterior horn. Hematoxylin-eosin stain. × 9.

N = neurinoma.

Fig. 40. Case 2, E.N. Cross section of nerve root indicated by arrow in Fig. 39. Hematoxylin-eosin stain. × 150.

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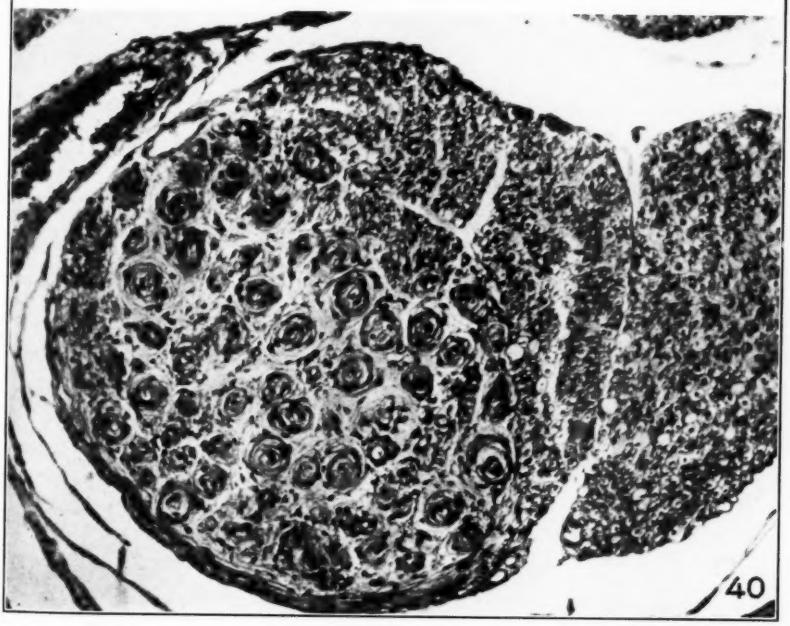

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Bailey and Herrmann Rôle of the Cells of Schwann

THE PATHOLOGY OF GRANULOMA VENEREUM *

RIGNEY D'AUNOY, M.D., AND EMMERICH VON HAAM, M.D.

(From the Departments of Pathology and Bacteriology of the Louisiana State Medical Center, and the State Charity Hospital, Louisiana, New Orleans)

Granuloma venereum, a disease widespread in tropical and subtropical countries, is best defined as an infectious granuloma of the pudenda. The question whether it is transmitted by sexual contact and should be considered a venereal disease is still undecided, but the experimental work of DeMonbreun and Goodpasture makes its traditional venereal nature appear at least doubtful.

In a previous study we discussed the incidence of granuloma venereum in various parts of the United States and, to some extent, were able to confirm Harris' statement that the disease seems to travel from the large seaports toward the inland sections along the great waterways. Supplementing Fox's report, we have collected 251 cases of granuloma venereum from the literature. This figure, however, is certainly not indicative of the true incidence of the infection in the United States, nor is our recent report of 294 cases observed over a period of 5 years in New Orleans a true gauge of the incidence of the disease in that community. Lack of cooperation on the part of afflicted patients, who generally belong to the lowest social strata, prevents exact diagnosis in many instances.

During studies of the various manifestations of the disease we have had occasion to examine not only surgically removed specimens and material from autopsied cases, but also, through the cooperation of the hospital staff, numerous biopsy specimens. It is the histopathological findings in this material that we wish to discuss in this commentation.

PATHOLOGICAL ANATOMY

The numerous classifications of the various lesions of granuloma venereum are based principally on their morphological character without consideration of their underlying pathogenesis. In our clinical study we have adopted, in a somewhat modified form, the purely descriptive terms applied to the lesions by Halty and have classified these as nodular, serpiginous, necrotic, hyper-

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trophic and cicatricial. From the standpoint of their pathological variations, however, we differentiate three groups of lesions: (1) those caused primarily by the infectious agent; (2) those caused principally as a result of a peculiar tissue reaction of the host to the infection; and (3) those caused by complications following the infection.

The nodular and serpiginous types of lesions, which were observed in 157 or 53.4 per cent of our cases, belong to the first pathological group. The nodular lesions are usually only the beginning stage of infection and undergo further changes which lead to development of the serpiginous ulcer, the most common and most typical manifestation of the disease. The lesion is characterized by a soft, easily bleeding granulation tissue which breaks through the epithelial lining of the skin and mucous membrane and shows a remarkable tendency towards superficial spread along the moist folds of the inguinal and pudendal regions (Manson). These lesions are only a few mm. deep and usually show very little suppuration. The exudate is serosanguineous, contains the infectious agent and spreads the disease by auto-inoculation. The rapidity with which the infection spreads varies considerably and healing may be observed in some portions of the lesion while other parts show continuous encroachment upon sound tissue. During the course of the disease considerable areas of the skin and mucous membranes are usually covered with these ulcerative lesions, with resultant severe anatomical mutilation of the genitalia and the perineum. The healing process is slow and the scars produced are atrophic with partial depigmentation of the skin and permanent loss of pubic hair.

The second group of pathological manifestations is the result of a peculiar host reaction to the infectious agent leading to hypertrophic and keloid-like lesions. These were present in 82 or 27.9 per cent of our cases. The surfaces of the hypertrophic lesions may be compared to the relief map of a mountainous country, with depressions between areas of piled up "mountains of tissue" (Harris). In consistence the lesions are firm and rather elastic, the overlying skin usually showing scars of previous ulcerations. There is often very little difference between this type of lesion and the true cicatricial or keloid-like lesion. In the latter there is an apparent overproduction of firm indolent tissue which replaces the

ulcerations. Our attention was first called to this type of lesion by complaints of patients that the scars from previous ulcerations were inclined to spread with gradual involvement of healthy parts in the keloid-like process. There is a distinct difference between this "spreading" type of scar and the usual atrophic and shrunken scar seen following ulcerative lesions. Our suspicion that we were dealing not with a healed stage of the disease but with a progressive lesion was further confirmed by the fact that histological examination showed the presence of Donovan bodies in the small nests of inflammatory cells embedded in the dense collagenous fibrous tissue obtained from such scars. Both the hypertrophic and the cicatricial (keloid-like) lesions show an excessive fibroblastic response of the host to the infection, usually developing rather early and inclined to progression. We believe that this lesion, observed rather commonly in the negro race, is due to an unexplainable constitutional peculiarity of the patient and not to chronic lymphatic obstruction, as claimed by Daniels. A further difference between the hypertrophic and keloid-like variety of granuloma venereum is found in the amount of intercellular collagenous substance present. In the hypertrophic form the amount is relatively small as compared to the mass of newly formed fibrocytes, while in the cicatricial lesion it is abundant.

The third group of pathological manifestations of granuloma inguinale consists of the lesions that occur as complications of the primary infection. The most frequent of such is secondary infection with an aerobic or anaerobic pyogenic or saprophytic genus. The onset of a virulent secondary infection is usually characterized by the appearance of toxic constitutional symptoms which are completely absent in the uncomplicated forms of granuloma venereum and by progression of the ulcerative process with the production of deep severe necrosis of soft tissues and even bone. During this stage Donovan bodies are generally not demonstrable, but there is an abundant mixed flora of secondary bacterial invaders. Here, the healing process results in severe mutilation of the genitalia with usual permanent impairment of their function. Fifty-five cases or 18.7 per cent of our series showed such deep ulcerations with necrosis and phlegmonous extension into the surrounding tissue. Two presented extragenital lesions, 1 case showing deep necrosis of the mouth and the structures of the neck with

secondary bronchopneumonia, the other a phlegmon of the gluteal region.

Histopathology

Fifty-six biopsies and 3 autopsies furnished the material for histopathological studies. In 3 cases a series of biopsies was obtained, enabling study of the evolution of the disease. Tissues were fixed in formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin, Wright's Gram's, and Giemsa's stains, and also by Mallory's aniline blue collagen stain.

In the very early or nodular type of granuloma venereum the epithelial lining of the skin does not appear to be interrupted, but there is distinct hypertrophy of the epithelium with offshoots from the papillae into the subcutaneous tissue (Gage). There is some edema in the papillary layers and infiltration of the corium of the skin, with polymorphonuclear leukocytes, eosinophiles and large monocytes (endothelial cells); these show no characteristic arrangement but seem embedded in a rather edematous matrix. With bacteria-revealing stains, numerous intracellular inclusion bodies — the so-called Donovan bodies — can be noted in the plasma of the large endothelial cells. On account of the pressure caused by infiltration the epithelium of the affected areas becomes thinned out and atrophic, exudate seeping through before the epithelial continuity is actually interrupted. Rapid proliferation of capillaries in the area of infiltration marks the beginning of the development of granulation tissue, which soon breaks through the epithelial lining of the skin to form the typical serpiginous lesions. Plasma cells, diffuse and in small groups, become increasingly prominent with progression of the lesion, and the leukocytes, which previously seemed to be the most important primary cellular response, are now found only at the surface. Large endothelial cells with numerous intracellular Donovan organisms are profuse between the capillary loops of the granulation tissue and are probably identical with the large foam cells described by Goldzieher and Peck as characteristic of this stage of the disease.

As the healing progresses, fibrocytes, which primarily were only sparsely scattered between the capillaries of the granulation tissue, become more abundant and, from the epithelial islands which have remained intact during the process of granulation tissue formation,

re-epithelialization of the surface begins. The scar tissue that repairs the serpiginous ulcer of granuloma inguinale usually shows a narrow epithelial lining with loss of all special structures of the skin and a moderate degree of subepithelial fibrosis. In the deep ulcerative processes, extensive necrosis with suppuration and phlegmonous extension may be noted. At the bottom of the necrotic areas there is sometimes fibrosis with interspersed nests of plasma cells and monocytes containing Donovan bodies. Bacterial stains reveal an abundant flora including *Borrelia vincenti* and fungi. Histological examination of the hypertrophic and the keloid-like lesions reveals marked fibrosis with numerous small nests of plasma cells and endothelial cells. The epithelium appears normal in thickness or shows slight hyperplasia. The walls of the larger vessels are thickened and their lumens narrowed. The lymph vessels are sometimes slightly dilated, but they are never the site of inflammatory changes as seen in elephantiasis caused by lymphogranuloma inguinale. The collagenous substance is extremely abundant in the hypertrophic cicatricial lesions. In the small collections of inflammatory cells Donovan bodies can be found, thus giving evidence of the activity of the lesion.

The Causal Agent

Although granuloma venereum has been known for over half a century, its causal agent has not yet been definitely established. Formerly identified with lues (Maitland, MacLennan), rhinoscleroma (Goodman), tuberculosis (LeDantec), and various other infections, since 1904 granuloma venereum has been definitely linked with an organism described by Donovan as Piroplasma and considered by him protozoal in nature. Martini in 1913, and Aragao in 1917, cultivated this organism on Sabouraud's medium and Aragao gave it the name "Schizomycete kalymmatogranulomatis." Castellani and Mendelson succeeded in growing an encapsulated bacillus that showed a close morphological relation to the Donovan organism and which was identified culturally as belonging to the group of *Aerobacter aerogenes*, genus *Encapsulatus*, Castellani and Chalmers. However, they do not believe this to be the causal organism. Aragao denied the identity of any bacillus belonging to the group of *Klebsiella* with the *Calymmatobacterium*, believing that the latter has never been cultivated. Organisms not

definitely classified but showing characteristics similar to the ones obtained by Castellani were grown by Poindexter, and by Goldzieher and Peck. DeMonbreun and Goodpasture confirmed Castellani's findings by growing organisms belonging to the aerogenous group from human lesions. Although they produced the disease by injecting human material into monkeys, they failed to do so with cultures of organisms obtained from such material. Campbell, in criticising the work of McIntosh, states very emphatically that, to date, no lesions have been produced with organisms cultivated from venereal granuloma. In the past year, Menon and his co-workers have critically analyzed the bacterial flora present in venereal granuloma. In addition to the Donovan organism, they have noted in human cases various spirochetes and fusiform bacilli, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Corynebacterium diphtheriae* and numerous types of staphylococci. These Indian investigators have succeeded in isolating the Donovan organism in pure culture from 12 out of 14 cases, and believe it to be related to *A. aerogenes*. Its inoculation in young rats and mice resulted in the production of distinct pathological lesions. Although the majority of recent authors seem to agree that an organism belonging to the *Klebsiella* group can be recovered from a large percentage of lesions present in granuloma inguinale, its etiological significance is still debated.

We have been able to observe the Donovan organism in practically all tissue sections stained by Wright's method in 60 to 80 per cent of smears obtained from the lesion. The nucleus of the capsulated body resembles a small curved bacillus, shows one or two terminal swellings simulating polar bodies and may, therefore, readily appear as "diplococcoid bodies" (Randall, Small and Belk). In acute fulminating lesions the majority of the organisms are not encapsulated and can easily be recognized extra- and intracellularly. In the large mononuclear cells they may fill vacuolar spaces in small clumps or clusters, or may be present in such numbers as to obliterate completely the outline and structure of the cells. We have regularly found plastin bodies, as described by Goldzieher and Peck, and they have been of considerable help in diagnosis, although we do not understand their significance. Our attempts to cultivate an organism from the human lesions have been successful in 8 out of 11 cases. The organism, similar to that

isolated by DeMonbreun and Goodpasture, belongs to the aerogenes group, but we have failed, so far, to produce any pathological lesions in laboratory animals comparable with the human lesion.

Summary and Conclusions

1. The pathology of granuloma inguinale has been studied in a series of 294 cases observed over 5 years at the State Charity Hospital of Louisiana at New Orleans.

2. The typical manifestations of the disease embrace nodular lesions and serpiginous ulcerations, which have a tendency to spread along the moist folds of the pudendal region, healing with the formation of atrophic scars.

3. Atypical manifestations are produced by unexplained increased fibroblastic reaction of the host leading to hypertrophic and cicatricial (keloid-like) lesions, which must be considered active stages of the infection.

4. Secondary infection produces serious ulcerative necrotic lesions which may severely mutilate the infected parts and give rise to sepsis and toxemia.

5. Histopathological study of biopsy material and tissue obtained at autopsy reveals that the stage of infiltration is quickly followed by the stage of granulation, during which the epithelial lining of the skin or mucous membrane is perforated by a vascular granulation tissue. Donovan organisms can be demonstrated in the infected tissue during all stages of the infection.

6. Search for a causal agent has resulted in the isolation of an organism belonging to the *Klebsiella* group. Inoculation of various laboratory animals with this organism has failed to incite lesions comparable to the disease in the human, although such lesions have been reported as produced by inoculation of material derived from human cases.

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DESCRIPTION OF PLATES

PLATE II

FIG. 1. Male negro, aged 25 years. Multiple nodular lesions of 3 weeks duration.

FIG. 2. Male negro, aged 32 years. Bilateral serpiginous ulcers of 4 months duration.

FIG. 3. Male negro, aged 37 years. Cicatricial lesion with progressive mutilation of penis and scrotum of 3 years duration.

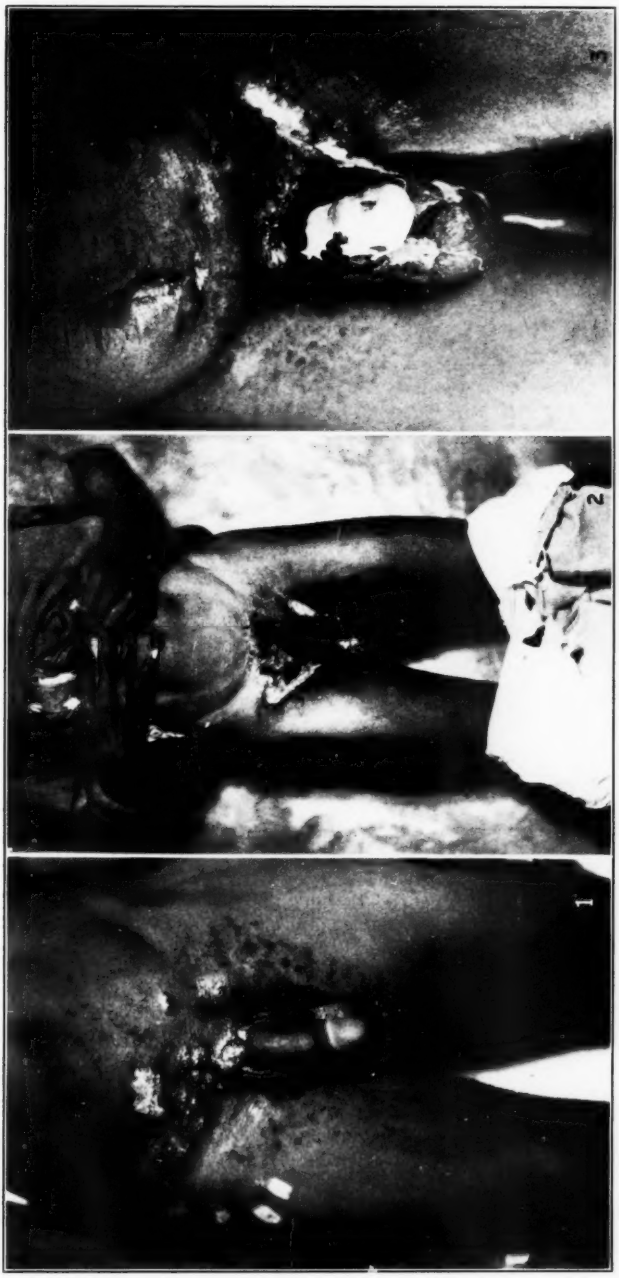

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PLATE 12

FIG. 4. Negress, aged 28 years. Hypertrophic lesion involving labia and mons veneris of 1 years duration.

FIG. 5. Negress, aged 52 years. Deep ulceration with necrosis of entire perineum of 8 months duration.

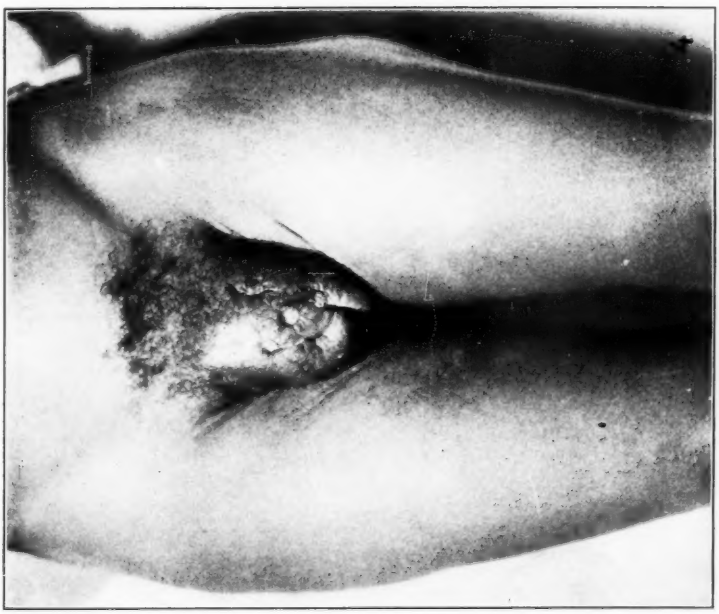

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PLATE 13

FIG. 6. Section through a nodular lesion showing initial proliferation of epithelium with subepithelial infiltration.

FIG. 7. Section through the margin of a serpiginous ulceration showing atrophy of the epithelium with marked edema and exudation.

FIG. 8. Section through the same lesion 3 weeks later showing development of a marked vascular granulation tissue.

FIG. 9. Section through a cicatricial lesion showing numerous active foci of the infection embedded in collagenous tissue.

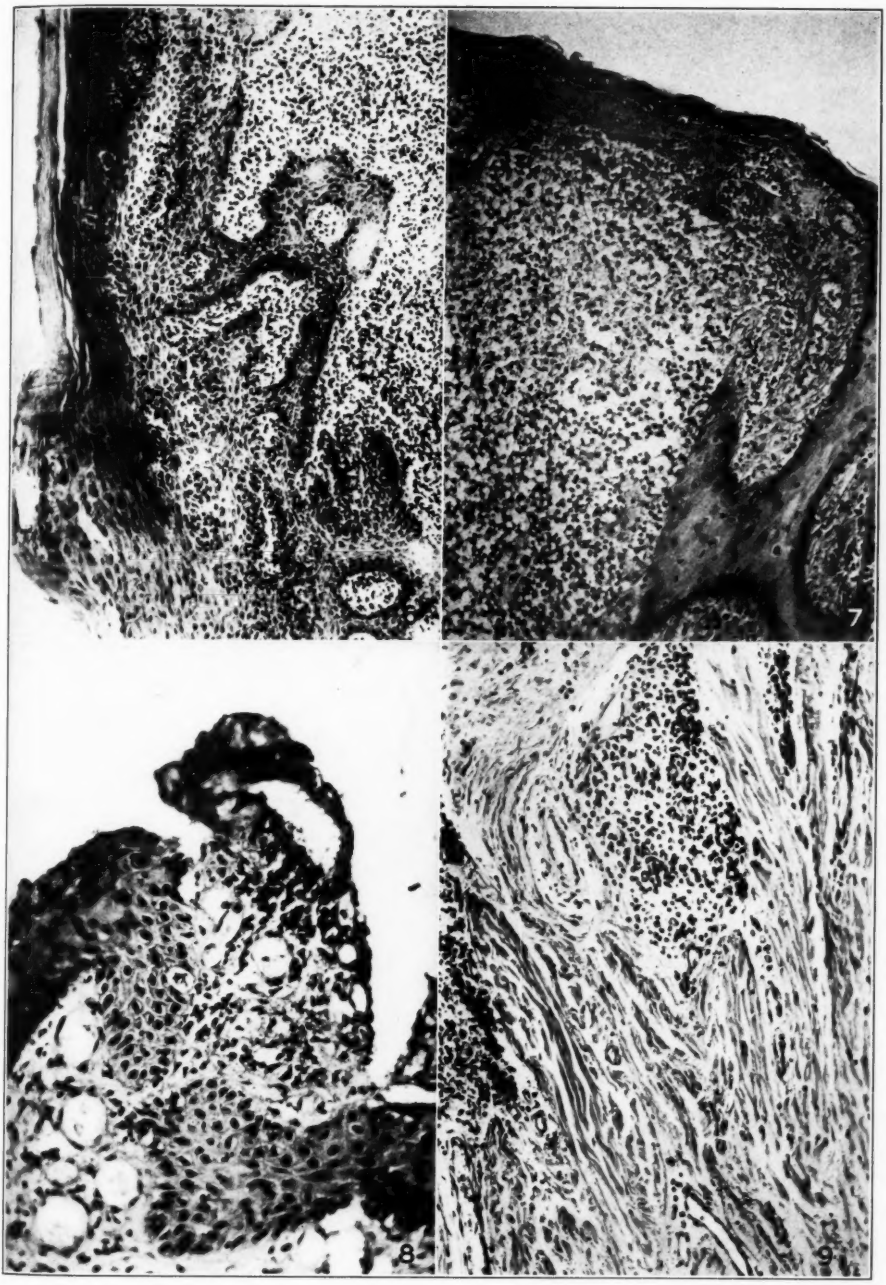

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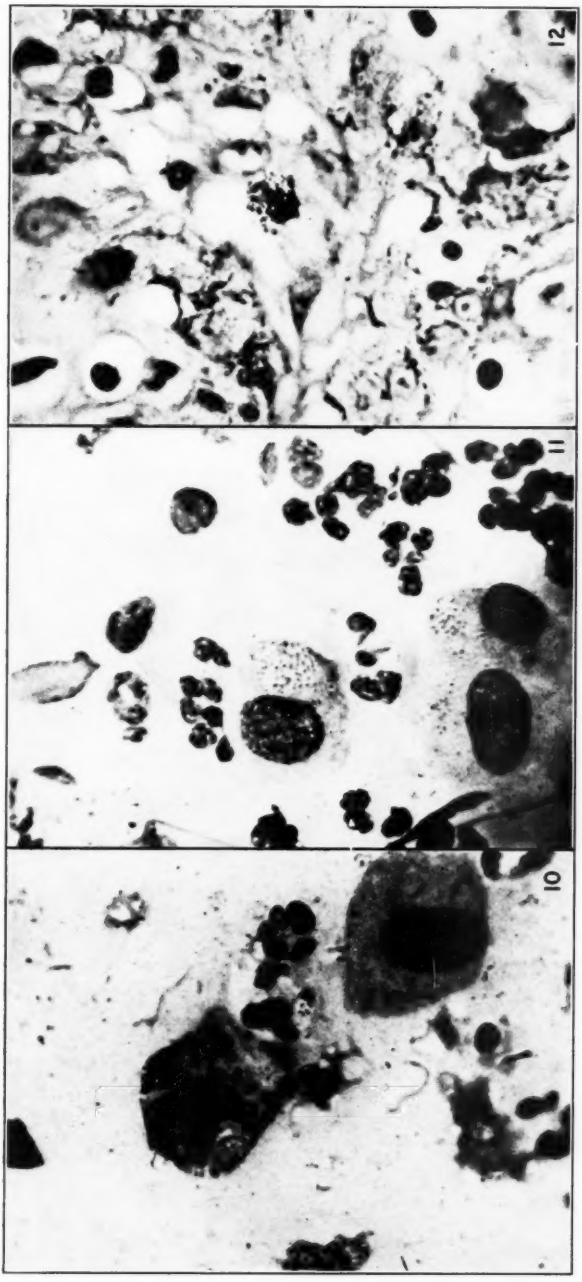
PLATE 14

FIG. 10. Smear from a serpiginous ulcer stained with Wright's stain. Numerous Donovan bodies are present in the plasma of a large monocyte.

FIG. 11. Smear from an acute ulcerative lesion stained with Wright's stain showing numerous non-encapsulated organisms growing in the plasma of monocytes.

FIG. 12. Section through a cicatricial lesion stained with Wright's method demonstrating numerous encapsulated and non-capsulated organisms in the plasma of an endothelial cell.



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PNEUMOCONIOSIS AND PULMONARY CARCINOMA *

ARTHUR J. VORWALD, M.D., AND JOHN W. KARR, M.D.

(From the Saranac Laboratory and the Roentgen-Ray Laboratory, Edward L. Trudeau Foundation, Saranac Lake, N. Y.)

The increasing prominence of pneumoconiosis and the more common recognition of primary pulmonary carcinoma have led to a large number of current reports of tumor development in the lungs of individuals who have been exposed to industrial dusts. The assumption has followed that inhaled dust plays an important rôle in the etiology of carcinoma.

Much notoriety, even in this country, has attended the high incidence of pulmonary carcinoma in men working in the mines in Schneeburg, Germany, and St. Joachimstal, Czechoslovakia.[1-3] The etiology of this type of carcinoma, although obscure, was for some time attributed to the dust these miners inhaled. In a recent critical review of these cases, however, Saupe [4] concludes that the newgrowth is due to radioactive substances in the air of these mines rather than to the inorganic dust. This interpretation is not generally recognized in the numerous publications that cite these cases as a foundation for their thesis that inhaled dust is a causative factor in the development of primary pulmonary carcinoma.

Many cases of carcinoma of the lung in persons exposed to inhalation of different dusts in occupation have been described in the recent literature. They are cited in the following table:

AUTHOR	CASES REPORTED	AUTHOR	CASES REPORTED
Allen [5]	2	Klotz and Simpson [15]	1
Cramer [6]	1	Lynch and Smith [16]	1
Dreyfus [7]	3	Maxwell [17]	1
Dible [8]	2	Middleton [18]	3
Egbert and Geiger [9]	1	Olson [19]	2
Fine and Jaso [10]	1	Sladden [20]	2
Frommel [11]	29	Saupe [21]	2
Gloyne [12, 13]	3	Sweaney *et al.* [22]	1
Harris [14]	4	Stewart and Faulds [23]	1

Some of these authors maintain that carcinoma of the lung in conjunction with pneumoconiosis is comparatively rare. There are those, however, who definitely affirm that inhaled dust is a chronic irritant and as such is a causative factor in the develop-

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ment of the newgrowth. Influenced perhaps by this latter view, the statement is often made in reporting a series of cases of primary pulmonary carcinoma that a given percentage gave a history of dust inhalation. Dust is thereby indirectly, if not directly, assigned an etiological significance.

Before any such generalizations are made, the following points should be proved: (1) that the incidence of pulmonary tumor in individuals with prolonged inhalation of a particular dust is significantly higher than in the general population; and (2) that the dust in question is irritating to the pulmonary parenchyma and is actually capable of producing proliferation and carcinomatous transformation of epithelial tissue.

The purpose of this study is to inquire into these conditions as they are manifested in roentgenological surveys of individuals exposed to industrial dusts; in postmortem observations on cases of pneumoconiosis; and in experimental animals that have inhaled dust over long periods of time.

Roentgenological Surveys

Summary of Literature: The roentgenographical reports on the lungs of 57,362 individuals exposed to dust in various industries throughout the world have been collected from the literature. The

Table I

Incidence of Pulmonary Tumors in Clinical and Roentgenological Examinations of Individuals Exposed to Dust, as Reported in the Literature

Number of cases	Non-silicotics	Silicotics	Pulmonary tumor
57,362	45,156	12,206	3 (0.005%)

interpretations as to the presence of nodulation or of neoplasm are presented in Table I. It is to be noted that pulmonary tumor was mentioned in only 3 of the entire group.

Saranac Laboratory Surveys: A study is in progress on individuals exposed to dust in a wide variety of occupations. The results of the surveys thus far completed are given in Table II.

Of the group with nodulation, only 1 individual showed roentgenological evidence of pulmonary tumor. He was 64 years of age and had worked in iron mines for 43 years.

Of the group without nodulation, 2 individuals had developed pulmonary tumor. Their ages were 59 and 68 years respectively. Both had worked in iron mines, the former for 27 years and the latter for 35 years.

In presenting these surveys we realize the difficulties that might attend a roentgenographical diagnosis of pulmonary tumor in silicotic individuals. The same degree of certainty does not obtain as with biopsy or postmortem examination, but lacking these sources of verification it is without question the most accurate method available. For a review of this aspect of the problem reference may be made to the papers of Hirsch [24] and Frothingham.[25]

TABLE II

Incidence of Pulmonary Tumors as Revealed by Serial Chest Roentgenograms in Individuals Exposed to Dust and Examined in the Saranac Laboratory

Occupation	Number examined	Pulmonary tumor
Iron mines	7,324	3
Foundries	6,613	0
Cement plants	823	0
Gypsum mills and plants	762	0
Copper mines	65	0
Silicotics	1,357	1 (0.074%)
Non-silicotics	14,230	2 (0.014%)
Total	15,587	3 (0.019%)

POSTMORTEM OBSERVATIONS

These observations are of particular significance, for the clinical and roentgenological interpretation of a case may be biased by a history of dust exposure. On examination at autopsy, however, many of these individuals fail to show evidence of pulmonary damage. Some dusts on inhalation into the lung produce no tissue change and are classified as inert. Others are active and cause a marked reaction. Of these, only silica, as Gardner [26] has repeatedly demonstrated, is capable of exciting a characteristic tissue response. This response may be modified by the presence of certain inert substances, such as carbon or iron, or may be complicated by infection, which is usually tuberculous. These differing properties of dust are often neglected by those who maintain that

there is a causal relation between pneumoconiosis and malignancy.

Summary of Literature: The anatomical studies on 444 individuals exposed to various types of dust have been collected. The lungs of these individuals were variously classified as pneumoconiotic, anthracotic, silicotic, anthraco-silicotic, and so on, according to the type of dust inhaled. They have been compiled in Table III.

TABLE III

Malignant Changes Seen at Autopsy in Individuals Exposed to Harmful Dusts, as Reported in the Literature

Number of cases	Malignant changes	Non-pulmonary	Pulmonary	
			Number	Per cent of all autopsies
444	10	4	6	1.3

Important observations from autopsy studies on silicotic individuals have been reported by the Miner's Phthisis Medical Bureau in South Africa.[27] The postmortem incidence of primary pulmonary carcinoma in miners dying from all causes during the years 1920–1925 inclusive, has been compiled in Table IV. This incidence is compared with that in European males never underground and presumably with no history of dust inhalation. The latter is from the statistics of the Johannesburg General Hospital, for the same period.

TABLE IV

Primary Carcinoma of the Lung Seen at Autopsy in European Miners and European Males, as Reported by the Miner's Phthisis Medical Bureau of South Africa, 1935

	Total number of autopsies	Carcinoma of lung	Per cent of all autopsies
European miners with silicosis	1438	10	0.70
European miners without silicosis	1679	12	0.71
European males never underground	1393	13	0.93

From these figures it is apparent that pulmonary carcinoma is a rare complication of silicosis in South African gold miners.

Saranac Laboratory Observations: Anatomical studies have

been made on the lungs of 178 males exposed to a variety of industrial dusts for periods of from 1 to 46 years. These studies are presented in condensed form in Table V.

In the entire group there were only 2 males with primary pulmonary carcinoma. One was 59 years of age and had worked in hematite mines for 27 years. The anatomical diagnosis with reference to the pathological changes in the lung caused by the inhaled dust was siderosis without evidence of fibrosis or nodulation. The other individual was 69 years of age and had mined hard coal for 18 years. The pneumoconiosis was interpreted at autopsy as anthraco-silicotic in type.

TABLE V

Incidence of Carcinoma Seen at Autopsy in Individuals Exposed to Dust and Examined in the Saranac Laboratory

Number examined	Years exposed	Silicotics	Carcinoma	
			Pulmonary	Non-pulmonary
178	1–46	136	2 (1.12%)	4

In the remaining 176 cases without pulmonary carcinoma, proliferation of the respiratory epithelial elements was not observed. This obtained even though pulmonary dust fibrosis was often massive and had compressed many epithelial-lined air passages (Figs. 1 and 2).

EXPERIMENTAL OBSERVATIONS

The experiments were designed to elicit the irritative capacity of the dust and its influence on tubercle bacillus infection. Various species of animals were exposed to heavy concentrations of different kinds of dust for long periods of time. The results are compiled in Table VI.

At autopsy, those animals that were exposed long enough showed deposits of dust in the lungs. Where the dust was silicious there was an associated proliferation of connective tissue, which in the case of pure silica resulted in the formation of localized hyaline fibrotic nodules. Often these nodules had formed in lymphoid aggregates contiguous to the mucosa of the major air passages (Fig. 3). In many instances the nodules surrounded and compressed a small epithelial-lined bronchiole. Within the nodules there were

usually one or more small slit-like air spaces lined with low cuboidal epithelium (Fig. 4). Under these circumstances there was every opportunity for irritation to the epithelium by the inhaled dust.

Only 2 animals, both guinea pigs, showed evidence of epithelial proliferation by developing small benign adenomas in the parenchyma of the lung. One guinea pig was exposed to ferruginous chert dust for a period of 749 days; the other had been kept in a soft coal tipple for 390 days.

TABLE VI

Incidence of Pulmonary Tumors Seen at Autopsy in Animals that have Inhaled Dust in the Saranac Laboratory

Type of dust	Guinea pigs	Rabbits	Rats	Chickens	Mice	Cats	Pulmonary tumor
Chalcedony	242	28			40		0
Quartz	894	41	201	12	24	4	0
Marble + quartz	25						0
Hematite + quartz	28						0
Quartz + gypsum	88		27		6		0
Granite	396						0
Chert	196 *	3	52		10		1
Hematite	106		31				0
Asbestos	235						0
Soft coal	37 *						1
Fluorspar — crude			15				0
Carborundum	213						0
Marble	172						0
Gypsum	200		12				0
	2832	72	338	12	80	4	2
Total			3338				0.06%

While none of the inhalation experiments was primarily planned to study the influence of dust on the development of pulmonary carcinoma, one experiment was designed to discover whether or not any strain of a given species was more susceptible than others. For this purpose the reaction to inhaled dust in ordinary albino mice was compared with that in a carcinoma-susceptible strain. There were 40 of the latter exposed to chalcedony over a period of 3 to 12 months. None of these animals manifested an unusual degree of epithelial proliferation in the lungs.

An objection to the interpretation of these experiments might be that strains of animals were employed that were not susceptible

to epithelial proliferation. In 2 guinea pigs, at least, this was not the case, for primary adenomas of the lung were discovered. In the total number of guinea pigs acquired from various sources it seems hardly probable that these should have been the only ones capable of responding by tumor formation when the proper stimulus was applied. If inhaled dust had been such a stimulus it would seem most likely that more of this rather large group should have responded. If, on the other hand, it is proper to perform carcinoma experiments on strains of animals in which spontaneous tumors are infrequent, no one can infer that such tumors have been a common occurrence in this series, since the incidence for the entire group is only 0.06 per cent.

Summary and Conclusions

If inhaled dust is of etiological significance in the development of primary pulmonary carcinoma, these two conditions should obtain: the incidence of pulmonary tumor in pneumoconiotic individuals should be higher than in the general population; and the dust in question should be irritating to the pulmonary parenchyma and actually capable of producing proliferation and carcinomatous transformation of epithelial tissue.

A compilation of our observations from roentgenological, postmortem and experimental studies shows that these conditions do not obtain.

Roentgenological observations, as compiled from the literature on the lungs of 57,362 males exposed to occupational dusts, revealed only 3 cases with primary pulmonary carcinoma, or an incidence of 0.005 per cent. The Saranac Laboratory surveys, which comprise stereoscopic chest roentgenograms of 15,587 males exposed to dust, showed an incidence of 0.019 per cent.

Postmortem examination of 3739 individuals exposed to dust, as compiled from the literature and from our own series, revealed 30 individuals with pulmonary carcinoma, or an incidence of 0.8 per cent. This incidence is lower than that reported in routine autopsy examinations of the general population.

Experimentally, of 3338 animals exposed to many different kinds of dust for long periods of time, only 2 guinea pigs revealed the presence of a pulmonary neoplasm. The tumors in both cases were similar and were interpreted as benign adenomas. All other

animals failed to show evident irritation, hyperplasia or tumor transformation of the epithelium lining the respiratory passages. This was observed irrespective of the activity of the dust, whether it was inert or had caused marked fibrosis of the pulmonary connective tissue.

Inhaled dusts, therefore, except those containing recognized carcinogenic substances such as radium and tar, cannot in general be considered as etiological factors in the development of primary pulmonary carcinoma.

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DESCRIPTION OF PLATE

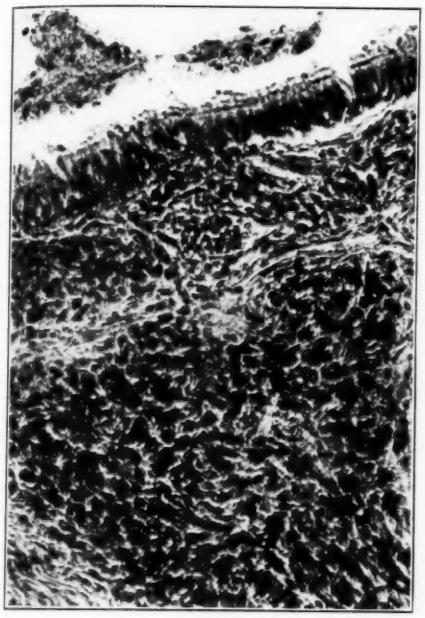
PLATE 15

FIG. 1. Section from the lung of a male, aged 55 years, who had worked in sandstone for 35 years. The section shows the bronchial mucosa covered with a thin layer of dust. There is also marked dust fibrosis in the submucosal connective tissue that is in contact with the basement membrane of the mucosa. Note the absence of epithelial hyperplasia. × 210.

FIG. 2. Section from the lung of a male, aged 44 years, who had worked in granite for 12 years. The small bronchiole is surrounded and compressed by fibrotic reaction to the inhaled dust. Even under these conditions the epithelium fails to show evidence of irritation or hyperplasia. × 210.

FIG. 3. Section from the lung of a rabbit that had inhaled quartz dust for 395 days. There is no evidence of irritation to the bronchial epithelium, even though extensive dust fibrosis has occurred in the submucosal connective tissue. × 210.

FIG. 4. Silicotic reaction in the parenchyma of the lung from the same animal. The air spaces in and about the silicotic nodule are lined with epithelial cells which show no evidence of irritation or hyperplasia. × 210.



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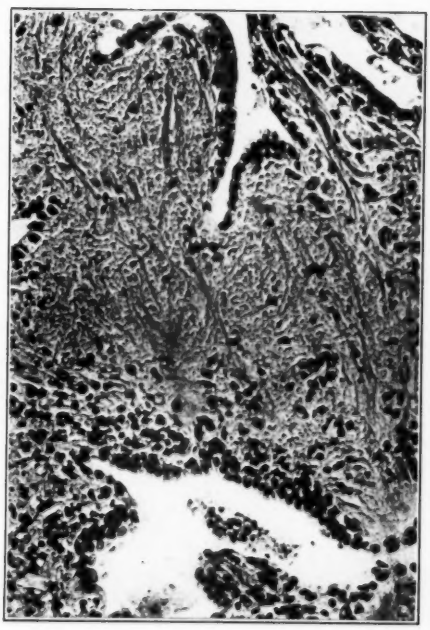

4

Vorwald and Karr

Pneumoconiosis and Pulmonary Carcinoma

TUBERCULOUS MENINGITIS AND ITS RELATION TO TUBERCULOUS FOCI IN THE BRAIN *

DAVID BERES, M.D., AND THEODORE MELTZER, M.D.
(From the Laboratories of The Mount Sinai Hospital, New York, N. Y.)

In the course of the past 17 years a number of cases of tuberculous meningitis have come under observation in the neuropathological laboratory of the Mount Sinai Hospital. It was noted that in each case the lesion was not limited to the leptomeninges but extended to involve the brain, assuming the character of a meningoencephalitis. In some instances, however, tuberculous lesions were found in the brain substance almost to the entire exclusion of any lesion in the meninges. On several occasions a systematic investigation of the tuberculous lesions and their interrelations was begun, but its completion was deferred until a larger number of cases and better preserved material would be available.

Until very recently it was thought that tuberculous meningitis usually followed the hematogenous dissemination of tubercle bacilli from some focus in the body. This concept was supported by many observations made on brains affected by tuberculosis. When viewed grossly such a brain showed the presence of a massive exudate in the interpeduncular space which tended to spread along the pial vessels in the sulci toward the dorsal surface of the cerebral hemispheres. Histological sections showed considerable alterations in the structure of the blood vessels, consisting of perivascular infiltration and extensive panarteritis with marked narrowing of the lumen. There were also caseation and necrosis.

In 1903 Trevelyan [1] reported that he found among 114 brains affected with tuberculous meningitis 23 that contained tuberculous masses. In addition, he had 10 brains that also contained tuberculous masses but which were not the seat of meningitis. He stated that it was possible for the cerebrospinal fluid to become infected by tubercle bacilli discharged from a focus adjoining the subarachnoid space. In 1924 Kment [2] examined the chorioid plexus of 27 cases of tuberculous meningitis and found tubercles in 60 per cent. He expressed the opinion that the leptomeningitis was in many

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cases dependent on the formation of tubercles in the chorioid plexus. In 1929, and again in 1933, Rich and McCordock [3] reported the results of a study of a large number of cases, on the basis of which they concluded that the concept of the hematogenous route of infection of the cerebrospinal fluid was untenable. They disagreed with Kment's deductions with regard to the tubercles in the chorioid plexus and, in turn, suggested that tuberculous meningitis was due to the rupture of a tubercle adjacent to the subarachnoid space or ventricular cavity, with consequent infection of the cerebrospinal fluid. They traced its origin from such sources in 75 out of 82 cases of tuberculous meningitis. In only 1 case did they consider the chorioid plexus as the source of the meningitis. In 1936 Ragins [4] reported the results of an investigation of 39 cases of tuberculous meningitis. Contrary to the reports of Rich and McCordock, this author found in 7 cases only indications that older caseous lesions in the brain or meninges had caused the diffuse meningeal infection. From the foregoing it is obvious that the problem of the genesis of tuberculous meningitis is still unsolved and hence the following observations may be considered pertinent.

Material

The material that formed the subject of this paper consisted of 30 brains. There were 95 additional cases which were not included because the material was not preserved in sufficient quantity to satisfy our demands. All of the 30 cases under consideration were the seat of tuberculous meningitis or tuberculomas. All brains affected with meningitis were cut by the guillotine method into slices about 5 mm. thick. Figure 1 shows the minimum number of sections of each brain that were cut. The brains revealing no tubercles were resectioned into thinner slices 1.5 to 2 mm. thick. As they were cut they were carefully examined for the presence of tubercles or other abnormalities. Sections were taken routinely of the interpeduncular space, the chorioid plexus and the ependyma. In surveying the material histological alterations in the meninges, the cerebral blood vessels, the cerebral substance, the ependyma and the chorioid plexus were examined and the postmortem findings in the other organs were investigated.

Anatomical Observations

Certain of the 30 cases studied exhibited collectively some common pathological features, permitting the arrangement into 5 groups as follows: *Group I:* This consisted of 14 cases showing a diffuse, exudative tuberculous meningo-encephalitis without tubercles in the cerebral substance. *Group II:* This consisted of 3 cases displaying, in addition to the diffuse tuberculous meningo-encephalitis, a few solitary tubercles in the cerebral substance that were not in contact with either the ventricular lining or the subarachnoid space. *Group III:* In this division were collected 6 cases, which in addition to the tuberculous meningo-encephalitis present, showed tubercles in the cerebral substance in contact with the leptomeninges. *Group IV:* This was composed of 5 cases which, together with the diffuse tuberculous meningo-encephalitis, presented numerous cortical and subcortical tubercles. *Group V:* This consisted of 2 cases of tuberculoma with the brain free of a diffuse, exudative tuberculous meningitis. The relation of these groups to each other and of the meningeal process to the cerebral lesions formed the basis of this study (Table I).

Group I: In the first group there were 14 cases in which there was a diffuse, exudative meningo-encephalitis of a tuberculous nature. Serial sectioning of the brain did not disclose the presence of tubercles in the cerebral substance. In all of the cases the inflammatory process was not limited to the meninges but tended to extend into the cortex by way of the extensions of the perivascular spaces, resulting in focal areas of secondary encephalitis and encephalomalacia often associated with hemorrhage. In some instances the affected areas displayed slight perivascular accumulations of lymphocytes with congestion of the blood vessels (Fig. 2). In others, there were also foci of necrobiosis (Fig. 3). These lesions, ranging from perivascular accumulations to necrobiosis, can be considered as successive events in one and the same pathological process. In all of the sections the intimate relation of the lesions to the blood vessels indicated that their development was the result of either direct hematogenous dissemination of tubercle bacilli or extension of the meningeal process along the pial vessels. The absence of cortical tubercles in the cases in this group, which formed almost half of the material, offered definite evidence that tuberculous meningitis may develop by other means than the dis-

TABLE I

Analysis of Anatomical Findings in the Brains in 28 Cases of Tuberculous Meningitis and in 2 Cases of Tuberculoma without Meningitis

Serial No.	Post-mortem No.	Age of patient	Presence or absence of tuberculous meningitis	Presence or absence of tubercles or lymphocytic accumulations in the chorioid plexus	Presence or absence of tubercles in the cerebral substance. If present, location or number of tubercles is stated	Presence or absence of tubercles in contact with the leptomeninges
Group I						
1	9685	5 yrs.	Present	Normal plexus	Absent	Absent
2	9819	19 "	Present	Normal plexus	Absent	Absent
3	9326	32 "	Present	Normal plexus	Absent	Absent
4	D3	—	Present	Normal plexus	Absent	Absent
5	66/4	3 yrs.	Present	Tubercle	Absent	Absent
6	10286	3 "	Present	Tubercle	Absent	Absent
7	9684	3 "	Present	Tubercle	Absent	Absent
8	9582	20 "	Present	Tubercle	Absent	Absent
9	9249	21 "	Present	Tubercle	Absent	Absent
10	9231	26 "	Present	Tubercle	Absent	Absent
11	9097	33 "	Present	Tubercle	Absent	Absent
12	9104	1½ "	Present	Lymphocytic accumulation	Absent	Absent
13	10060	27 "	Present	Lymphocytic accumulation	Absent	Absent
14	10276	52 "	Present	Lymphocytic accumulation	Absent	Absent
Group II						
15	9537	5½ yrs.	Present	Lymphocytic accumulation	Basis pontis and left frontal lobe	Absent
16	8147	24 "	Present	Normal plexus	Rt. frontal lobe	Absent
17	9540	1¾ "	Present	Tubercle	Brachium pontis and left frontal lobe	Absent

Group III						
18	9296	16 mos.	Present	Tubercle	Island of Reil	Present
19	7799	5 yrs.	Present	Lymphocytic accumulation	Rt. occipital lobe	Present
20	6762	19 "	Present	Lymphocytic accumulation	Cerebellum	Present
21	10173	43 "	Present	Normal plexus	Cerebrum	Present
22	6808	9 "	Present	Tubercle	Rt. occipital lobe	Present
23	8217	9 "	Present	Lymphocytic accumulation	Cerebellum	Present
Group IV						
24	9933	15 mos.	Present	Normal plexus	18 tubercles	Present
25	5026	2 yrs.	Present	Normal plexus	56 tubercles	Present
26	9378	5 "	Present	Tubercle	10 tubercles	Present
27	6993	8 "	Present	Section of plexus lost	110 tubercles	Present
28	9638	42 "	Present	Lymphocytic accumulation	30 tubercles	Present
Group V						
29	7858	36 yrs.	Slight	Tubercle	Frontoparietal and Rt. occipital lobe	Present
30	9381	50 "	Absent	Normal plexus	Cerebrum	Present

charge of bacilli from a cortical lesion. The pathological changes described in the cerebral substance appeared to be secondary to the meningeal process or else a coincidental development.

Group II: This group consisted of 3 cases in which, along with the diffuse tuberculous meningo-encephalitis, tubercles were found that were not in contact with either the ventricular lining or the pia mater. These cases differed from those in the first group only by the presence of these solitary tubercles. In Case 9537 there was one tubercle at the basis pontis and another in the gray matter of the left occipital lobe. In Case 8147 there was a tubercle in the white matter of the right frontal lobe. In Case 9540 there was one tubercle in the brachium pontis and another in the left frontal lobe, at the junction of the white and gray matter (Fig. 4). The remote location of these tubercles makes it improbable that they were the source of the infection of the subarachnoid space. Hence it is permissible to conclude that the meningitis in these cases was not the result of the presence of these tubercles.

Group III: This group contained 6 cases in which, in addition to the tuberculous meningo-encephalitis, there were found tubercles in contact with the ependyma or leptomeninges. It will be recalled that such lesions were regarded by Rich and McCordock [3] as the foci from which tubercle bacilli entered the cerebrospinal fluid. It would seem that most of these lesions, in themselves, do not offer any evidence either for or against such a hypothesis. However, since in the entire series these were the only cases that might fit in with such a theory, they will be described in detail. In Case 9296 there was a tubercle 4 mm. in diameter in the gray matter at the Island of Reil. The caseous process extended directly into the adjacent sulcus. Figure 5 which was taken from this lesion shows, in addition to the tubercle, two smaller areas of infiltration in the cortical tissue. It is not possible by any positive means to determine whether such a lesion was the result or the cause of the meningeal process, or whether it developed simultaneously with the latter. In Case 7799 there was a tubercle 3 mm. in diameter at the base of a sulcus in the right occipital lobe. The meningeal reaction at the site of the lesion was minimal (Fig. 6). In this case, also, one cannot determine from the available evidence which was the primary lesion, the tubercle or the meningitis. In Case 6762 there was a conglomerate tuberculous focus in the cerebellum

occupying an area 1 cm. in diameter. The inflammatory process extended into the neighboring sulci (Fig. 7). In Case 10173 there were a few instances of caseous tubercles in contact with the leptomeninges (Fig. 8). In Case 6808 there was a large tubercle (tuberculoma), 5 by 3 cm., which was situated in the right occipital lobe occupying the space between the descending horn of the lateral ventricle and the ventral aspect of the lobe. The last case, 8217, presented a large tuberculoma measuring 1.5 by 3.5 cm. On the opposite side there were two adjacent tuberculomas, together measuring 2.5 by 1 cm.

Group IV: This group consisted of 5 cases, characterized by the presence of numerous tubercles throughout the brain (Fig. 9). The brain findings in these cases, in the multiplicity of the lesions, resembled closely the lungs, spleen and kidneys in instances of generalized miliary or disseminated tuberculosis. It may reasonably be assumed that these lesions were the result of direct hematogenous dissemination and thus a correlation between the distribution of such tubercles and that of the vascular supply to the brain may be of interest. There was a total of 224 tubercles in the 5 brains in this group. The right and left side of each brain were affected to nearly the same extent. When classified in relation to the particular artery involved, it was found that 51.8 per cent of the tubercles were in the area vascularized by the middle cerebral artery, 33 per cent in the zone supplied by the anterior cerebral artery, and 15.2 per cent in parts supplied by the posterior cerebral artery. The cortical system of arteries accounted for the vast majority of the lesions, as compared to the very small number of lesions in zones vascularized by the central system. This is in keeping with the fact that the cortical system nourishes a volume of cerebral tissue far in excess of that supplied by the latter. When the tubercles were tabulated according to whether they were situated in the cortical or subcortical areas of the brain, it was found that the former contained 40 per cent of the lesions, the latter 38 per cent, while 22 per cent were situated at the junction of the cortex and the subcortex. The tubercles varied in size from 1 to 3 mm., although in some instances many were so close together as to form fairly large conglomerate masses. To cause such generalized dissemination large numbers of tubercle bacilli must have been circulating in the blood stream, and it is likely that the meningeal

infection, present in all the cases in this group, was due to the hematogenous dissemination, developing at the same time as the tubercles in the cerebral substance.

Group V: This group includes 2 cases of tuberculoma without meningitis, which indicates that tuberculous meningitis need not follow the presence of a tuberculous focus adjoining the ventricular cavity or subarachnoid space. The first, Case 7858, was that of a female, aged 36 years, who was subjected to an exploratory craniotomy for a neoplasm in the left cerebral hemisphere. Six days afterward she developed an elevation of temperature and the spinal fluid, which was previously normal, contained 656 cells per cmm. She died 2 weeks later. The brain disclosed 2 tuberculomas. One was 2 cm. in diameter and was situated in the left parietotemporal region, adjacent to the descending horn of the lateral ventricle. The ependymal lining between the tuberculoma and the ventricle showed evidence of disintegration without actual loss of continuity. The second lesion was only 5 mm. in diameter and was situated at the base of a sulcus in the right occipital lobe (Fig. 10). The pia arachnoid membrane was not thickened at the site of this lesion although there was an exudate in the adjacent sulcus. The chorioid plexus showed early tubercle formation. The brain displayed no diffuse exudative tuberculous meningitis, despite the fact that in some sections there were a few large and small round cells in the subarachnoid space. Case 9381 was that of a 50 year old male negro who was operated upon on two occasions for an expanding intracranial lesion. The brain, when sectioned, revealed the presence of 3 tuberculous lesions. One was situated in the cortex of the brain and measured 4 by 3.5 cm. It was in direct contact with the subarachnoid space. The meninges were thickened and adherent to its surface. In the right cerebral hemisphere there was a second lesion measuring 3 by 4.5 cm. The inflammatory process extended into the neighboring sulcus but there was no meningeal reaction (Fig. 11). In the right occipital lobe there was a third lesion measuring 0.5 cm. in diameter. There was no exudate at the base of the brain. Miliary tubercles were found in the lungs, liver, spleen and kidneys. The cases in this group are instances in which caseous lesions were situated in direct contact with the leptomeninges and yet, diffuse exudative tuberculous meningitis was absent.

The Chorioid Plexus

In the series studied there were 11 cases in which tubercle formations were found in the chorioid plexus. In an additional 8 cases histological examination revealed accumulations of cells of the small and large round cell type. All of the tubercles were microscopic in size. Although they were in direct contact with the circulating cerebrospinal fluid, thus enhancing the probabilities of the latter becoming infected by such tubercle bacilli as might be discharged, yet the extremely small size of the tubercles leads one to question whether or not they could discharge sufficient numbers of bacilli to cause the widespread, exudative reaction usually seen at the base of the brain. At best, it can be said that the tubercles and cellular accumulations are indicative of the presence of bacilli circulating in the blood stream.*

Tuberculous Meningitis Affecting the Spinal Cord

In 3 of the cases studied the subarachnoid space around the spinal cord was the seat of a tuberculous exudate as marked in degree as that found at the base of the related brain. Sections of the spinal cord revealed areas in the nerve trunks that were the seat of inflammatory reaction, though they were not in direct anatomical continuity with the subarachnoid space. There was a marked lymphocytic infiltration in the connective tissue between the nerve bundles and among the nerve fibers (Fig. 12). The infiltration was most dense around the blood vessels. In 1 case there were giant cells and tubercle formation in the nerve roots. While the infection here could readily extend to the pia arachnoid space enveloping the nerve roots, its ultimate spread to the endoneurium and perineurium was most likely hematogenous in origin, and due to extension by means of the small blood vessels which ramify in the connective tissue surrounding the nerve bundles.

Relation of Tuberculous Meningitis to Tuberculous Infection in Other Organs of the Body

One of the arguments that has been advanced against the hematogenous theory is that tuberculous meningitis does not occur in

* A finding which apparently has no bearing on this problem but which may be mentioned in passing is that in 53 per cent of the cases psammoma bodies were found in the chorioid plexus.

all cases of miliary tuberculosis; also, that cases of meningitis may take place without a generalized miliary tuberculosis. One hundred cases of tuberculous meningitis in which there were complete postmortem examinations were surveyed. A comparison of the findings in the organs of the body with those in the brain was made. It was noted that the spleen was affected in 76 per cent of the cases, the lungs in 69 per cent, the liver in 64 per cent and the kidneys in 50 per cent. These figures differ to a slight extent from those published by other authors (Paterson,[5] MacGregor, Kirkpatrick and Craig,[6] and Blacklock and Griffin [7]). In this series the spleen was affected in a greater percentage of cases than is usually reported. Organs such as the pancreas, thymus, adrenals and thyroid, were affected in only a small percentage of cases. The meninges apparently belong in the same category as the organs just mentioned. Thus, for reasons not understood, tuberculous meningitis need not develop in all cases of disseminated and miliary tuberculous disease.

Rich and McCordock have concluded that other serous cavities such as the pleural, pericardial and peritoneal, behave with regard to tubercles in a manner similar to that which they postulate for the meningeal space, claiming that, in such cavities, rupture of a tubercle adjacent to the cavity discharges its contents, resulting in an exudative inflammation. This is out of accord with the findings recorded in the postmortem protocols of 100 completely autopsied cases of tuberculous meningitis which were surveyed by us. In this series miliary tubercles on the pleural lining were found in 12 per cent, on the pericardial lining in 7 per cent, and on the peritoneum in 10 per cent. In nearly all of the cases the cavities were normal. In a few instances the peritoneum showed localized, caseous fibroblastic thickening surrounding tuberculous ulcers of the intestines. This again leads one to conclude that the presence of a tubercle in the wall of a serous cavity does not necessarily result in a tuberculous infection of that cavity.

Comment and Summary

The anatomical survey of 28 cases of tuberculous meningo-encephalitis and of 2 cases of tuberculoma without meningitis resulted in the following observations:

In a large proportion (14 cases) there was a diffuse, exudative tuberculous meningo-encephalitis without the presence of tubercles

in the cerebral substance. As part of the inflammatory process there were, in the cortex, small foci of encephalorrhagia, perivascular infiltration, lymphocytic accumulations and necrobiosis. These changes were in relation to the blood vessels.

A small proportion (3 cases) showed, in addition to the diffuse tuberculous meningo-encephalitis, a few solitary tubercles in the cerebral substance which were not in contact with either the ventricular lining or the leptomeninges. It is unlikely that they could have served as foci from which the meningitis developed.

In a small quota (6 cases) there were, in addition to the tuberculous meningo-encephalitic process, tuberculous lesions in the cerebral substance, which were in contact with the leptomeninges. The lesions in 4 of these cases were cortical tubercles. The remaining 2 cases in this group revealed large tuberculomas giving clinical manifestations of a cerebral neoplasm.

In another group (5 cases) there were numerous solitary cortical and subcortical tubercles in the cerebral substance. These were present along with the diffuse, tuberculous meningo-encephalitis. The great number of tubercles suggests that they were the result of direct blood stream dissemination.

In 2 cases tuberculomas in contact with the ventricular lining or with the leptomeninges were present without a concurrent, diffuse exudative meningitis.

The evidence presented shows that in all cases of tuberculous meningitis there was an extension of the inflammatory process into the cortex resulting in foci of encephalitis, which varied in degree from perivascular infiltration to tubercle formation. In a few cases solitary tubercles were found but these were not in contact with the ependyma or leptomeninges. When tubercles occurred in great numbers they were due to direct hematogenous dissemination. Large solitary tuberculomas occurred with or without meningitis. In only 6 cases in this series were cortical tubercles demonstrated which might have been responsible for the coexistent meningitis. In 11 cases the chorioid plexus disclosed the presence of tubercle formation. The blood vessel changes which were observed during the course of study revealed no findings that differed from those already described by other observers.

NOTE: Our appreciation is due to Dr. Joseph H. Globus, under whose supervision the work was performed, for his advice and helpful criticism during the course of this work.

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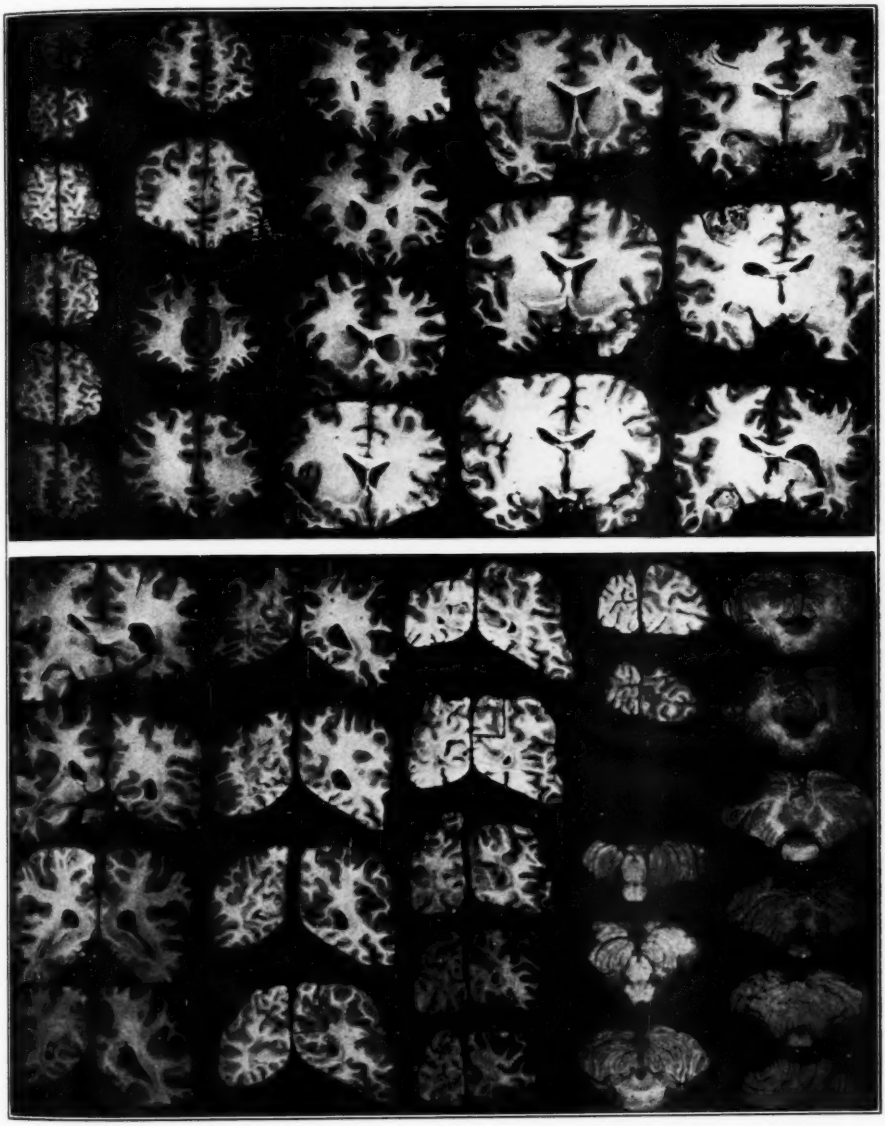
DESCRIPTION OF PLATES

Plate 16

Fig. 1. Photographs illustrating the minimum number of sections cut from each brain.



1

Beres and Meltzer Tuberculous Meningitis

PLATE 17

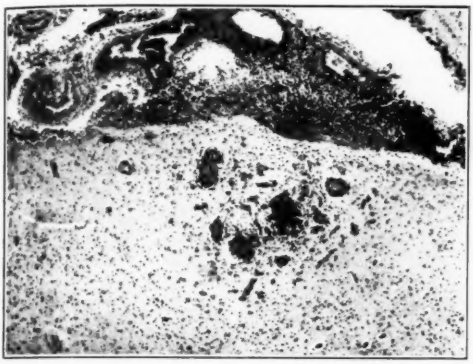
FIG. 2. Case 10060. Section showing tuberculous exudate in subarachnoid space with perivascular foci of lymphocytes in cortex. H & E stain.

FIG. 3. Case 10060. Section showing exudate in subarachnoid space with cortical focus of necrobiosis and lymphocytic accumulation. H & E stain.

FIG. 4. Case 9540. Section showing tubercle at the junction of white and gray matter, remote from leptomeninges. H & E stain.

FIG. 5. Case 9296. Section showing tuberculous focus at the base of a sulcus. H & E stain.

FIG. 6. Case 7799. Section showing tuberculous focus at the base of a sulcus with area of encephalomalacia. Note minimal meningeal reaction. H & E stain.



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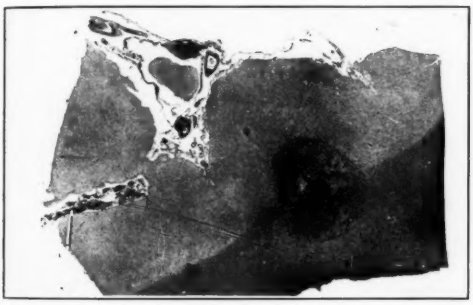

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Beres and Meltzer Tuberculous Meningitis

Plate 18

Fig. 7. Case 6762. Section showing conglomerate tuberculous focus in cerebellum. H & E stain.

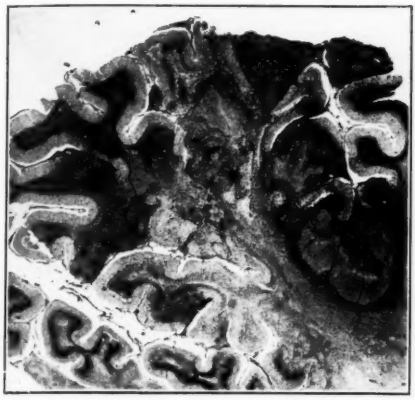
Fig. 8. Case 10173. Section showing tubercle in contact with leptomeninges. H & E stain.

Fig. 9. Case 6993. Photograph illustrating numerous solitary tubercles in cerebral hemisphere. There were 110 tubercles in this brain.

Fig. 10. Case 7858. Photograph illustrating tubercle adjacent to a sulcus. No diffuse exudative meningitis was present.

Fig. 11. Case 9381. Section showing tuberculoma (T) in contact with leptomeninges. No diffuse exudative meningitis was present. H & E stain.

Fig. 12. Case 9685. Section of spinal nerve trunk showing a giant cell (arrow) and lymphocytic infiltration into the connective tissue. H & E stain.



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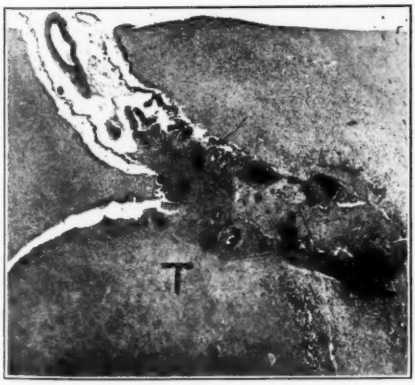

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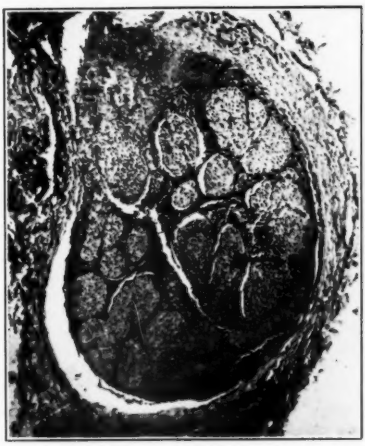

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Beres and Meltzer

Tuberculous Meningitis

AN EXPERIMENTAL STUDY OF COMPLEMENT AND HEMOLYTIC AMBOCEPTOR INTRODUCED INTO CHICK EMBRYOS *

ALICE POLK, A.B., G. J. BUDDINGH, M.D., AND E. W. GOODPASTURE, M.D.

(From the Department of Pathology, Vanderbilt University Medical School, Nashville, Tenn.)

Recent investigations in this laboratory and elsewhere have shown that a number of viruses will infect the tissues of embryo chicks.[1, 2] We have utilized the same technique to study bacterial infection, demonstrating that many microorganisms pathogenic for man will likewise infect the chick embryo and cause pathological lesions in several respects quite similar to those observed in the human host.

It is a striking fact that as yet no convenient experimental hosts have been found in which infectious lesions, analogous to those encountered in many specific diseases of the human being, can be induced with pure cultures of the respective bacterial incitants. This applies, for example, to such familiar diseases as typhoid fever, diphtheria, epidemic meningitis, gonorrhea, soft chancre, *H. influenzae* infections, whooping cough and others.

In order that studies of the pathogenesis and immunology of such infections may be facilitated it is desirable that experimental hosts be utilized in which lesions comparable with those observed in the respective human diseases may be readily and conveniently reproduced.

Preliminary studies in this laboratory have indicated that the chick embryo can be utilized for investigations of this character, and more extensive research might demonstrate that this sterile living host has a very broad applicability to the study of such problems.

For example, Goodpasture and Anderson have shown that the chick embryo can be infected with pure cultures of such bacteria as staphlococcus, streptococcus, *E. typhi*, *C. diphtheriae*, *Br. abortus*, among others; and that some lesions simulate those encountered in the natural hosts.[3] More recently Buddingh and Polk [4]

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have reported that experimental infection with meningococci simulates that in man, and Gallavan,[5] and Gallavan and Goodpasture [6] have described lesions in embryos infected with *H. influenzae* and *H. pertussis* which correspond quite closely to those occurring in man.

Because of the evident value of the chick embryo technique for the study of infectious diseases, it has appeared quite likely that this host might advantageously be used also for investigations concerning phenomena of susceptibility, resistance and immunity.

There are certain obvious advantages that the chick embryo offers for the study of infection. It is, of course, an organized living host whose tissues are sterile. It is a relatively uniform medium because it has not yet been subjected to infective disease and consequent immunity reactions, or to dietary changes, maturation and other incidents of independent life in the exterior environment. It is of course an accessible, cheap and convenient experimental host.

Because chick embryos have been found to be susceptible to infection by a number of human pathogens, it seems evident that they might be utilized for investigations not only of natural protective responses, but as passive vehicles to evaluate the significance of experimentally introduced antibodies in the serums of immune animals and other substances designed to influence the course of infection. The proved protective effect of diphtheria antitoxin introduced into the embryo against infection with *C. diphtheriae* is an illustration.[3]

In an attempt to develop a satisfactory method for these purposes it seemed advisable to investigate such matters as the presence in the chick embryo of native agents that are considered to play a part in combating infection, for example complement, and the fate of artificially introduced complement and antibodies.

In the first place we tested embryonic material for the presence of complement, using an antisheep amboceptor system. No native complement being demonstrated with this system, we introduced guinea pig complement and followed its distribution and fate. We then introduced antisheep amboceptor by various routes, because of its relative stability and ease of demonstration, and followed its distribution in the fluids of the host. Finally complement and amboceptor were introduced into embryos to circulate in the blood concurrently.

By these experiments we wished to determine among other things the practicability of introducing similar foreign substances into the embryo in quantities adequate to the study of their effects in later investigations of active and passive resistance of the embryo to specific infectious agents.

Natural Complement in Chick Embryos

Rywosch [7] found no lysin for rabbit erythrocytes in the serum of chick embryos before the 21st day. She failed likewise to demonstrate complement. She stated, however, that bacteriolysins for *E. coli* were present in extremely small amounts from the 14th day onward. Sherman [8] found that lysins for rabbit erythrocytes did not appear until the 21st day of incubation, when the chick was pecking through the shell. Complement for rabbit but not for sheep erythrocytes was found in small amounts in embryos on the 17th day of incubation.

For our work we chose first to test the presence of complement for sensitized sheep cells in serum, cavity fluids and tissues of the embryo, and in the serum of recently hatched chicks and young and adult chickens.

Technique for Collecting Serum and Fluids

Adult and half-grown chickens were bled from the wing vein with a needle and syringe. Blood of newly hatched and embryonic chicks was aspirated directly from the exposed heart. Chorio-allantoic and amniotic fluids were collected by puncturing the respective membranes with capillary pipettes and aspirating the fluids. Tissue extracts were prepared by pooling the heart, lung, spleen, liver and kidneys of several embryos. These tissues were ground in a mortar and diluted with twice their volume of 0.85 per cent saline. All the materials to be tested were centrifuged to obtain a clear supernatant fluid.

Test for Complement: Sheep cells sensitized with rabbit anti-sheep hemolysin were used to test for complement. The reagents were set up in the following proportions:

Antisheep hemolysin diluted to contain 1 unit in	0.1 cc.
Washed red cells (sheep), 2 per cent	0.1 cc.
Salt solution, 0.85 per cent	0.3 cc.
Fluid to be tested for complement	0.1 cc.

With each experiment a positive control, using pooled fresh guinea pig serum for complement, was run as well as a negative salt solution control.

The tests were always performed within 1 to 2 hours after the fluids were collected. Typical results are recorded in Table I.

TABLE I

Complement Content of Chicken Serum

Serum dilutions	Guinea pig serum (control)	Serums of (6) adult chickens	Serums of (3) half-grown chickens	Serums of (3) 2-day-old chickens
No amboceptor (control)	–	–	–	–
Dilution 1 : 30	+++	–	–	–
Dilution 1 : 5	+++	+++	++	–
Dilution 1 : 0	+++	+++		++

+++ = complete hemolysis.
– = no hemolysis.

No complement whatever was demonstrated in undiluted serum, cavity fluids and tissue extracts of 17, 19 and 20-day embryos. Two-day-old chicks contain a small amount of complement. It is increased in amount in half-grown chicks and is quite abundant in adult fowls. This is in agreement with previous work.

Recovery of Guinea Pig Complement after Introduction into Chick Embryos

Complement Dropped onto the Chorio-Allantoic Membrane: On 3 successive days 0.4 cc., 0.1 cc., and 0.1 cc. respective amounts of fresh guinea pig serum complement were dropped onto the chorio-allantoic membrane of chick embryos 16 days old, exposed by the coverslip method.[9] Twenty-four hours after the last dose the embryos were killed and tested for complement. None could be demonstrated in the blood serum or organ extract, but it was present in the chorio-allantoic fluid in a concentration so that 0.1 cc. was sufficient to hemolyze completely one portion of sensitized sheep red corpuscles.

Complement Injected into the Body of the Embryo: Embryos of 16 days incubation received into their bodies by injection 2 cc. of fresh guinea pig serum. After 24 and 72 hours they were tested, but no complement was found in the blood serum or organ ex-

tracts. However, partial hemolysis of one portion of sensitized red sheep cells was produced by the chorio-allantoic and amniotic fluids 72 hours after the injection.

Complement Injected Intravenously: Injection of 0.2 cc. amounts of fresh guinea pig serum was made intravenously (technique follows) into embryos of 12 days incubation. After varying intervals the embryonic fluids were tested for complement. The results are recorded in Table II.

TABLE II

Demonstration of Complement after Intravenous Injection

Time after injection, (1) embryo each	Blood serum	Chorio-allantoic fluid	Amniotic fluid	Organ extract	Control complement *
5 min.	+++	–	–		+++
2 hrs.	++	–	–	+	+++
4 hrs.	++	–	–		+++
16 hrs.	+	++	–	+	+++
24 hrs.	–	++	–		+++

* The control complement was diluted 1:10 and kept at 37° C.

These experiments indicate that guinea pig complement in the quantities used disappears from the circulation in about 16 hours. Complement placed on the chorio-allantoic membrane or injected directly into the body of the embryo, if absorbed, does not persist in demonstrable quantity in the blood serum after 24 hours. Although leakage into the chorio-allantoic fluid was not certainly eliminated there is a possibility that some complement was excreted into this fluid. Guinea pig complement seems to be fairly rapidly inactivated *in vivo* in these experiments.

Intravenous injection is the method of choice for assuring the presence of complement in the circulating blood for a few hours. After 16 hours it had almost disappeared from the serum, although it was still demonstrable after 24 hours in the chorio-allantoic fluid, into which it was probably excreted.

The degree of dilution of complement within the internal and external fluids of the embryo in these experiments is not known. The average egg used weighed about 60 gm. The total volume of the embryonic tissue fluids, and especially the total blood volume,

is relatively small, and it is evident from the experiments that the dilution in serum, at least for several hours, is within a range that permits complement to remain effective in the tests used.

Recovery of Antisheep Hemolysin after its Introduction into the Incubating Egg

Normal chick embryo serum contains no hemolytic amboceptor for sheep cells. Rabbit hemolysin having a hemolytic titer for

TABLE III

Recovery of Hemolysin after Dropping it onto the Chorio-Allantoic Membrane

Material tested	Embryo blood serum	Chorio-allantoic fluid	Amniotic fluid	Organ extract
Group 1. Pooled fluids of 10 embryos 24 hrs. after dropping 0.2 cc. of hemolysin in 0.8 cc. of saline	++	+++		–
Group 2. Pooled fluids of 6 embryos 24 hrs. after dropping 0.1 cc. of hemolysin in 0.9 cc. of saline	–	+++	–	–
Group 3. Pooled fluids of 3 embryos 72 hrs. after dropping 0.1 cc. of hemolysin in 0.9 cc. of saline	–	+++	–	–
Group 4. Pooled fluids of 7 embryos 24 hrs. after dropping 0.2 cc. of hemolysin in 0.8 cc. of saline	–	+++	+++	–
Group 5. Pooled fluids of 5 embryos 24 hrs. after dropping 0.1 cc. of hemolysin in 0.4 cc. of saline	–	+++	+++	–

+++ = complete hemolysis.
++ = partial hemolysis.
+ = slight hemolysis.
– = no hemolysis.

sheep cells not less than 1:1000 was used in these experiments. It was introduced into the chick embryo by the various methods described below and its presence or absence in the fluids and tissues was determined by testing their hemolytic power for sheep cells in the presence of fresh guinea pig complement. The materials to

be tested were collected and prepared by the same methods as those described under the complement determination. The materials for the test were mixed in the following proportions:

Fluid to be tested for hemolysin	0.1 cc.
Salt solution, 0.85 per cent	0.3 cc.
Washed red corpuscles (sheep)	0.1 cc.
Fresh guinea pig complement (0.1 cc. solution hemolyzes 0.1 cc. sensitized cells)	0.1 cc.

TABLE IV

Tests for Hemolysin after Injection into Yolk Sac

Material tested	Chick embryo blood serum	Chorio-allantoic fluid	Amniotic fluid	Organ extract
Group 1. Pooled fluids of (4) embryos 24 hrs. after injection of 0.2 cc. of hemolysin in 0.8 cc. of saline	±	+++	+++	–
Group 2. Pooled fluids of (4) embryos 48 hrs. after injection of 0.1 cc. of hemolysin	–	–	–	–
Group 3. Pooled fluids of (4) embryos 96 hrs. after injection of 0.1 cc. of hemolysin	–			–

Hemolysin Dropped onto the Chorio-Allantoic Membrane

Using the coverslip technique measured amounts of hemolysin were dropped onto the exposed chorio-allantoic membrane. The embryos were returned to the incubator at 37° C. and examined at varying intervals. The results are recorded in Table III.

Recovery of Hemolysin after Injection into Yolk Sac

The yolk sac of eggs containing 16 or 17-day-old chick embryos received by injection measured amounts of hemolysin, and tests for its presence were made after various intervals. The results are recorded in Table IV.

Recovery of Hemolysin after Injection into Albumin Sac at an Early Stage of Development

In this experiment a slit was cut in the small end of eggs containing 2, 3, 4 and 6-day-old embryos. Through the slit measured

amounts of hemolysin were injected into the albumin sac which lies at this end. The injection was made with needle and syringe. The holes were then sealed with paraffin and the eggs kept in the incubator until the 14th day of incubation when they were examined for hemolysin. The results are tabulated in Table V.

TABLE V

Tests for Hemolysin after Injection into Albumin Sac

Material tested	Blood serum *	Chorio-allantoic fluid	Amniotic fluid
Group 1. 2-day-old embryos injected with 0.2 cc. hemolysin	(1) –	–	+++
	(2) ±	–	+++
Group 2. 3-day-old embryos injected with 0.2 cc. hemolysin	(1) ±	±	+++
	(2) –	±	+++
	(3) ±	±	+++
Group 3. 4-day-old embryos injected with 0.2 cc. hemolysin	(1) –	±	±
	(2) –	–	±
Group 4. 6-day-old embryos injected with 0.2 cc. hemolysin	(1) –	–	–
	(2) –	±	+

* Each number refers to a different embryo.

The concentration of the hemolysin in the amniotic fluid is due probably to the opening of the sero-amniotic junction that occurs in the chick embryo about the 12th day when the proteins from the albumin are liberated into the amniotic fluid.

Recovery of Hemolysin after Injection into the Body of the Embryo

Fourteen to 16-day-old embryos were exposed by the coverslip technique. The chorio-allantoic and amniotic membranes were pierced and the hemolysin injected directly into the embryo by means of a needle and syringe. It was not always possible to be sure what part of the embryo received the hemolysin.

These results show that the hemolysin is absorbed into the blood stream from the body in most cases, but not all. In an effort to learn why the material introduced in this manner cannot always be recovered from the blood stream a number of injections of methylene blue were made in chick embryos from which the shells

had been completely removed so that the whole chick could be observed. The methylene blue could be seen through the thin skin of the embryo. It diffused through the tissues but could often be seen flowing back out of the hole made by the injecting

TABLE VI

Tests for Hemolysin after Injection into Bodies of Embryos

Material tested	Blood serum	Chorio-allantoic and amniotic fluid	Organ extract
Group 1. (6) 16-day-old embryos 24 hrs. after injection	+++	+++	+
Group 2. (3) 16-day-old embryos 48 hrs. after injection	++		±
Group 3. (6) 14-day-old embryos 92 hrs. after injection	+++	+++	–
Group 4. (7) 16-day-old embryos 96 hrs. after injection	+++		
Group 5. (8) 14-day-old embryos 24 hrs. after injection	±	+++	–
Group 6. (6) 14-day-old embryos 48 hrs. after injection	+	++	–
Group 7. (6) 14-day-old embryos 24 hrs. after injection	+++	+++	–
Group 8. (6) 14-day-old embryos 24 hrs. after injection	±	+++	
Group 9. (8) 14-day-old embryos 24 hrs. after injection	+++	+++	
Group 10. (5) 14-day-old embryos 24 hrs. after injection	+++	+++	
Group 11. (3) 14-day-old embryos 48 hrs. after injection	+++	+++	
Group 12. (4) 14-day-old embryos 72 hrs. after injection	–	+++	
Group 13. (4) 14-day-old embryos 72 hrs. after injection	++	+++	

needle. In one instance, after a supposedly intraperitoneal injection, the methylene blue flowed out of the mouth of the chick. A number of uncontrolled factors determine whether the injected hemolysin remains to be absorbed or flows out into the surrounding fluids.

Recovery of Hemolysin after Intraperitoneal Injection

One attempt to control the point of injection was made. The embryos were exposed by removing about a square inch of shell. Through this a small hole was made in the membranes and the leg of the chick was pulled through with sterile forceps. The injection was made by passing the needle through the firm part of the thigh into the peritoneum. The firmer tissues of the thigh kept the material from flowing out and the hemolysin was recovered from the

TABLE VII

Recovery of Hemolysin after Injection through Leg into Peritoneum

Material tested	Blood serum	Chorio-allantoic and amniotic fluid
Embryo 1. 15 days old, 24 hrs. after injection	+++	–
Embryo 2. 15 days old, 24 hrs. after injection	+++	++
Embryo 3. 15 days old, 24 hrs. after injection	++	–
Embryo 4. 15 days old, 24 hrs. after injection	+++	+
Embryo 5. 15 days old, 24 hrs. after injection	+++	+

blood stream in each instance where the embryo was 15 or more days old. But in the younger chicks the legs are too small and too soft to make the injection practicable. Another disadvantage of this method lies in the fact that the membranes were always considerably torn by pulling the leg through them.

Recovery of Hemolysin after Intravenous Injection

To make this injection the large vessels of the membrane must be accurately located by candling the eggs under a strong light. The membranal vessels may be distinguished from the deeper ones by the fact that the former are fixed in the membrane whereas the latter may be seen moving freely in the embryo fluids. An area about half an inch square is marked on the shell to indicate the position of the largest membranal vessel. At this point the eggshell is cut and opened by the coverslip technique. A tuberculin syringe with a 27 gauge needle is used for the injection. The direction of

the blood flow must be determined by slipping the needle under the vessel until the lumen is occluded. The vessel will collapse slowly on the side toward which the blood flows. Then the needle, held almost parallel to the vein, is slipped along in the direction of the current until the point enters the vessel. The egg may be tilted to secure a better position for the needle, if necessary. When the needle has entered, a little of the material forced from the syringe may be clearly seen through the transparent vessel wall, flowing

TABLE VIII

Tests for Hemolysin after Intravenous Injection

Time after injection *	Blood serum	Chorio-allantoic fluid	Amniotic fluid
5 min.	++++	++++	+
2 hrs.	++++	++++	++
4 hrs.	++++	++++	±
8 hrs.	++++	++++	+++
12 hrs.	++++	++++	++
24 hrs.	++++	++++	+
72 hrs.	++++		
6 days	++++	++++	++++

* Each interval refers to tests on 1 embryo.

in the direction of the blood. Arteries are more difficult to enter than veins, but this is not impossible. However, the membranal arteries are smaller than the membranal veins and the shell is therefore less apt to be opened at the position of an artery. The hemorrhage resulting from intravenous injections may sometimes be stopped by lifting the vessel with the needle to cut off the blood flow and searing the point of hemorrhage with a heated needle. This procedure requires some manual dexterity but can be acquired with practice. Usually the amount of blood lost is not significant.

By this method exact amounts of material can be introduced into the chick embryo, leaving the membranes practically intact. To keep a high percentage of the embryos alive after the injection of so much foreign protein, a few precautions should be taken. The material to be injected should be kept warm. The embryos should be returned to the incubator immediately after injection. About

0.05 cc. seems to be the most satisfactory amount for injection, although some serums seem to be more toxic than others. Five hundredths of a cubic centimeter equals approximately one twelve hundredth of the total weight of the egg.

Eight-day-old embryos received injections by this method with 0.05 cc. amounts of hemolysin and were tested at varying intervals. The results are tabulated in Table VIII.

Thus the experimental introduction of hemolytic amboceptor into the chick embryo has indicated that very little of this foreign antibody is absorbed so as to be demonstrable at the intervals used after dropping it onto the chorio-allantoic membrane or injecting it into the yolk sac.

Only after direct injection into the body of the embryo or into the membranal veins has hemolysin been recovered in significant quantity from the blood and extra-embryonic fluid.

Although a much simpler procedure technically, injection into the embryo itself has the disadvantage that it cannot easily be controlled, and it involves considerable damage to the chorio-allantoic membrane. This is especially disadvantageous should one wish to use the membrane subsequently for inoculation with an infectious agent.

By means of intravenous injection a stable antibody can be introduced into the chick embryo, and in the case of hemolytic amboceptor it can persist relatively undiminished for as long as 6 days. These experiments suggest that hemolytic amboceptor may be excreted, possibly by the kidneys, to gain access to the chorio-allantoic fluid.

Unlike complement, amboceptor, in our experience, seems to remain stable within the tissues of the embryo within the time limits employed, that is through 6 days. Although quantitative measurement has not been made, the tests performed indicate very little loss of amboceptor from the fluids and tissues as a whole.

Injection of Complement and Amboceptor into the Same Embryo

In order to study the combined effects of complement and antibody on infection induced in the chick embryo it would be necessary to introduce both substances simultaneously or at intervals into the same experimental host. It was important to determine in these preliminary experiments that both complement and antibody

can circulate in the blood stream in effective amounts and react together on the antigen under investigation.

To test this possibility hemolytic amboceptor was first injected intravenously, followed later by a similar injection of guinea pig complement. At intervals serum of the embryo was removed and tested for hemolysis against a suspension of sheep cells.

Intravenous Injection of Hemolysin and Complement into the Same Chick Embryos

Three 13-day-old chick embryos received intravenously 0.025 cc. of 1:1000 antisheep hemolysin. Twenty-four hours later each of these embryos also received an intravenous injection of 0.2 cc.

TABLE IX

Combined Intravenous Injection of Complement and Hemolysin

Time elapsed after complement injection	Blood serum	Amniotic fluid
5 minutes	+++	−
1 hour	++	±
2 hours	+	+

fresh guinea pig complement. The 3 chicks were killed in succession at intervals of 5 minutes, 1 hour and 2 hours. Blood serum and amniotic fluid of each were collected. To 0.2 cc. of each of these was added 0.3 cc. of 0.85 per cent saline and 0.1 cc. of 2 per cent red sheep cells. The results are shown in Table IX.

DISCUSSION

Our experiments have confirmed the observations of others that antisheep hemolytic complement is absent from the fluids and tissues of chick embryos. This complement makes its appearance within a day or two after hatching and gradually increases in concentration presumably until maturity.

Because of the absence of complement in the chick embryo, a host proved to be susceptible to a variety of pathogenic infectious agents, it might be possible to discover whether complement necessarily participates in a variety of protective reactions between the host and invading parasites, both by determining whether or not certain reactions take place in a host devoid of complement, and

by the passive introduction of complement preferably by intravenous injection.

The chick embryo has been found to be susceptible to certain infectious agents to which newly hatched chicks are immune. What part complement may play in this greater susceptibility is open to investigation.

It is still a question whether or not antitoxic and antibacterial reactions are in part dependent on the presence of complement; and since it has been shown that antibody contained in foreign serum and complement can be successfully introduced intravenously into embryos, the effects of specific antibodies, with and without complement, can be studied in this living host in relation to combating or preventing the infections to which they are proved to be susceptible.

In the case of the neutralization of diphtheria toxin by antitoxin complement does not seem to be at all necessary.[3] But the case might be quite different with antibacterial antibodies, and a relatively uniform host in which these important questions can be studied should be of considerable usefulness. Any direct experimental means of demonstrating and measuring the effectiveness of serums now in use for the prevention or amelioration of infectious disease would be of inestimable value.

It was with the idea of developing such a technique that the present investigation was undertaken. The results will be utilized in testing the effectiveness of antitoxic and antibacterial antibodies passively introduced into the embryo before and after infection with those pathogenic agents that induce in this living host lesions and other pathological phenomena similar to those encountered in natural hosts.

It seems evident that for testing the effectiveness of complement and antibody, whether singly or combined, the intravenous route of introduction of the desired materials is the technique of choice and offers the greatest possibilities. Under these circumstances complement is demonstrable in the circulating blood for several hours, and the more stable hemolytic antibody for several days. This would indicate that the effects of the presence of similar antitoxic or antibacterial antibodies can be determined under conditions of induced infection in this host.

Summary and Conclusions

1. Complement for sensitized sheep cells is not present in the serum, extra-embryonic fluids and tissues of the chick embryo before hatching. After hatching it is suddenly present and seems gradually to increase to a maximum in the adult fowl.

2. Neither hemolytic amboceptor nor complement appears regularly in the circulating blood of the chick embryo 24 hours after dropping them onto the chorio-allantoic membrane or after injection into the yolk sac or albumin sac.

3. Following injection of hemolytic amboceptor into tissues of chick embryos it was recovered from the circulating blood or extra-embryonic fluids after 72 hours.

4. Following intravenous injection into chick embryos, hemolytic amboceptor was demonstrated in the serum and extra-embryonic fluids of the embryo over a period of 6 days. Complement disappears in about 16 hours after this mode of injection and appears in somewhat diminished quantity in the chorio-allantoic fluid after its disappearance from the blood stream.

5. Amboceptor and complement, circulating concurrently after intravenous injection into a chorio-allantoic vein, were proved to be effective in hemolyzing sheep red cells for a period of at least 2 hours.

6. The method of intravenous injection as described is recommended for the study of the effect of immune substances on subsequent infections of the chick embryo.

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THE FAILURE OF ALLERGIC INFLAMMATION TO PROTECT RABBITS AGAINST INFECTION WITH VIRULENT PNEUMOCOCCI *

PAUL R. CANNON, M.D., AND GEORGE HARTLEY, JR., M.A.
(From the Department of Pathology, University of Chicago, Chicago, Ill.)

Although inflammation is generally regarded as a protective mechanism of great importance, opinion still differs concerning the actual part it plays in bacterial localization. Some workers believe that the localization is accomplished by the formation of a mechanical barrier from the rapid coagulation of plasma and thrombosis of lymphatic vessels. Therefore, unless the bacteria give off substances that hinder this formation, the effectiveness of the localizing process should vary directly with the speed and intensity of the inflammatory reaction, and allergic inflammation, because of its accelerated development and greater intensity, should tend to localize bacteria near the portal of entry more rapidly and more effectively. This mechanical "walling off" theory has been widely accepted, particularly in the field of tuberculosis, and the belief is general that allergic inflammation causes a more effective walling off of tubercle bacilli in reinfection.

Other workers question the primary importance of a mechanical barrier in bacterial localization. They suggest, rather, that the deposition of fibrin and the thrombosis of lymphatics is essentially a secondary phenomenon accompanying the inflammatory response. The primary factor in the localization is the interaction of the bacteria and the tissues whereby the bacteria either fail to grow and spread freely, or, as in acquired immunity, tend to adhere to one another and to the tissues. The inflammatory reaction, through the local accumulation of leukocytes and immune substances, helps to reinforce the process of bacterial localization and hinders the bacterial growth and spread through the tissues.

Disagreement concerning these two points of view may be due to different experimental methods or to the fact that a biological mechanism adequate in some situations may be totally inadequate in others. For example, an inflammatory reaction which may restrict the growth and spread of slightly virulent or invasive micro-

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organisms may fail to do so with highly virulent ones. Reliance on inflammation as a primary localizing mechanism presupposes its general utility, however, inasmuch as the nature and virulence of microorganisms cannot usually be foretold.

In many of the experiments that have been performed to ascertain the function of inflammation in bacterial localization, bacteria of various kinds were injected into well developed areas of inflammation. Such a procedure, however, cannot prove that inflammation effectively localizes bacteria because it does not show what happens in infections in essentially normal tissues; satisfactory proof would require its production at the time or shortly after the bacteria enter the tissues.

The present paper describes experiments performed to test the assumption that the inflammatory reaction, *per se*, can protect a susceptible animal (rabbit) against infection with a virulent microorganism (*Pneumococcus* type I), and to determine whether the intensified inflammatory reaction of allergy is comparable to the inflammatory response of acquired immunity in protecting an animal against an otherwise lethal infection.

Comparatively few studies have been reported with these questions in mind. Hanger [1] sensitized rabbits to egg albumin and observed that when a suspension of *Bact. lepisepticum* was injected into an area of hypersensitive inflammation, the resulting lesion was intense and "the animals practically always succumbed." When the same type of experiment was performed in rabbits immune to *Bact. lepisepticum*, the local lesions extended "to the border of the hypersensitive reaction, but no further."

Rich [2] found that if only a few fowl cholera bacilli were suspended in pneumococcal soluble specific substance and injected into the skin of rabbits allergic to it, the animals died of septicemia just as rapidly as did the normal controls. In other words, allergic inflammation developed too slowly to prevent bacterial spread and death of the animals. Bull and McKee [3] observed that if rabbits allergic to pneumococcus were infected intranasally with a broth culture of *Bact. lepisepticum*, followed later by the production of a local allergic inflammation in the nose by instillation of pneumococcic autolysate, the infection flared up and the animals died from a blood stream invasion by *Bact. lepisepticum*. These experiments all indicate that a violent allergic inflammation occurring at a portal

of entry may tend to disseminate the infectious agents rather than to localize them.

The experiments of Opie [4] on the nature of the Arthus reaction suggest that this phenomenon is due to the union in the tissues of antigen and antibody, accompanied or quickly followed by an accelerated inflammatory response which tends to keep the protein localized or fixed. If this interpretation is correct, it should be possible, by suspending virulent microorganisms in a solution of protein, to determine whether or not the accelerated inflammatory response of the Arthus reaction might also localize the bacteria. If allergic inflammation is an important primary mechanism of defense in early infection, such a procedure should demonstrate this fact. Also, its influence on the growth of the bacteria in the field of inflammation and on their spread to the body as a whole could be observed. With this object in mind the following experiments were performed.

Materials and Methods

Rabbits weighing from 1600 to 2000 gm. were prepared as follows: One group was sensitized to egg albumin by the subcutaneous injection, at intervals of from 5 to 7 days, of 1 cc. of a 5 per cent aqueous solution of powdered egg albumin. Positive Arthus reactions appeared usually after from 6 to 7 injections. These animals are referred to as allergics,* and were used in the experiments from 7 to 10 days after the last injection. A second group of animals was prepared similarly, but during the course of sensitization the rabbits were also immunized against the pneumococcus with a formol-killed culture of pneumococci or of a pneumococcic autolysate. These animals are referred to as allergic-immunes. A third group of rabbits was immunized against the pneumococci, but was not sensitized to egg albumin; these animals are called pneumococcus-immunes. The fourth group served as normal controls.

The pneumococci for the experiments were grown for from 6 to 8 hours in dextrose rabbit serum broth; sterile broth was added to the bacterial suspension so that dilutions of from 10^{-1} cc. to 10^{-8} cc. of the original culture were present in a volume of 0.5 cc. These

* The term allergy as used in this paper is employed in its broadest sense of altered reactivity with the locally accelerated development of hyperergic inflammation.

dilutions were then mixed carefully with equal volumes of 10 per cent egg albumin solution and 1 cc. of the mixture was injected subcutaneously. In some of the experiments the viable germ-content of the higher dilutions was determined by plating in blood agar (poured plate method) 0.5 cc. of the pneumococcic dilution. The *Pneumococcus* type I used was culture A5, which was obtained from Dr. O. H. Robertson.[5] This microorganism, under appropriate conditions, will kill rabbits almost invariably in a dilution of 10^{-7} cc.

The Comparative Protective Values of Normal and Allergic Inflammation and of Active Specific Immunity in Pneumococcic Infection

Series 1:

Ten rabbits allergic to egg albumin and 10 normal controls were injected subcutaneously with the albumin-pneumococcus mixture, the dilutions of the pneumococcic culture ranging from 10^{-2} cc. to 10^{-7} cc. The allergic animals all appeared healthy, being survivors of a group of 18 which had been injected with egg albumin over a period of several weeks. During this time 5 of the animals died, 1 failed to become Arthus-positive, and 2 were not used because of illness at the time of the experiment. These 10 survivors, therefore, were presumably somewhat hardier than the control animals with which they were compared. As a check on the influence of active immunity in general protection, 5 animals previously immunized with the pneumococcic vaccine were injected intravenously with dilutions of culture ranging from 10^{-3} cc. to 10^{-7} cc. The results of this experiment are shown in Table I.

The inflammatory lesions in the allergic animals were all visible within from 3 to 6 hours and at the time of death were edematous, indurated, and in most cases contained hemorrhagic centers. Those in the normal rabbits were typical of the dermal pneumococcic lesion, with a diffusely spreading edema and small hemorrhagic spots in the skin, but with no induration or focal hemorrhagic center. It is obvious that an intense allergic inflammatory reaction failed in every instance to prevent death, regardless of any part it may have played in favoring bacterial fixation at the portal of entry. In other words, inflammation had no appreciable influence on the ultimate outcome of the infection, either in time or degree.

On the other hand, the survival of 4 of the 5 immune animals after intravenous injection indicates the superiority of general specific immunity over local inflammation in a non-immune animal as a life-saving procedure.

TABLE I

The Comparison of the Lethal Effect Following the Subcutaneous Injection of Pneumococcus-Egg Albumin Mixture in Normal, in Egg Albumin-Allergic, and in Pneumococcus-Immune Rabbits

Animal No.	Dilutions of pneumococcus culture	Fate of animals: hours until death	Heart's blood cultures
Allergic			
1	10^{-2}	16	o
2	10^{-3}	36	+
3	10^{-3}	24	+
4	10^{-4}	24	+
5	10^{-4}	24	+
6	10^{-5}	24	+
7	10^{-5}	36	+
8	10^{-6}	48	+
9	10^{-6}	56	o
10	10^{-7}	36	+
Non-allergic			
11	10^{-2}	36	+
12	10^{-3}	24	+
13	10^{-3}	20	+
14	10^{-4}	18	+
15	10^{-4}	30	+
16	10^{-5}	20	+
17	10^{-5}	36	+
18	10^{-6}	18	+
19	10^{-6}	36	o
20	10^{-7}	24	+
Pneumococcus-immune			
21	10^{-3}	Lived	
22	10^{-4}	24	o
23	10^{-5}	Lived	
24	10^{-6}	Lived	
25	10^{-7}	Lived	

o = Cultures not taken.

Series 2:

A smaller group of 6 rabbits (2 allergic, 2 immune to the pneumococcus alone, and 2 normals) were injected subcutaneously with the albumin-pneumococcus mixture in which the pneumococci were in high dilution (10^{-5} cc. to 10^{-8} cc.). The allergic animals gave strong Arthus reactions 9 days before, and the pneumococcus-

immune rabbits had survived an intravenous injection of 10^{-4} cc. of pneumococcic culture a week previously. The results (Table II) show that only the animals immunized against the pneumococcus survived and in them the local lesion at the end of 24 hours was practically negligible.

In both series, therefore, allergic inflammation failed to protect the host by walling off the bacteria before they could enter the blood stream. The actively immunized animals, with one exception, remained healthy even when injected with from 10 to 100

TABLE II

The Comparison of the Lethal Effect from the Subcutaneous Injection of Pneumococcus-Egg Albumin Mixture in Normal, Egg Albumin-Allergic, and Pneumococcus-Immune Rabbits

Animal No.	Dilutions of pneumococcus culture	Fate of animals: hours until death	Remarks
Allergic			
26	10^{-7}	48	
27	10^{-8}	48	
Normal			
28	10^{-7}	48	
29	10^{-8}	48	
Pneumococcus-immune			
30	10^{-5}	Lived	Minimal local inflammation
31	10^{-6}	Lived	Minimal local inflammation

All animals injected subcutaneously.

times more pneumococci than the non-immunes. Furthermore, the local inflammatory lesions in the 2 immune animals were minimal in size at the time that the normal and allergic animals died. There was no correlation between the extent or intensity of the local inflammation and the protection of the host. Practically, therefore, neither the vigor of the inflammatory reaction nor its ultimate effect in immobilizing the bacteria would seem to matter if ultimately the animal dies from an overwhelming bacteremia. Evidently, in any situation in which the escape of only a few virulent bacteria may lead to a fatal outcome, little confidence can be placed in inflammation as a primary determining element in a mechanism of defense.

The Protective Effects of Existing Inflammation in Normal, Allergic and Immune Rabbits Infected with Virulent Pneumococci

Inasmuch as many workers have attempted to determine the value of inflammation as a localizing mechanism by the inoculation of bacteria into previously prepared areas of inflammation, we next injected small numbers of virulent pneumococci directly into areas of developing inflammation produced in normal, albumin-allergic, and pneumococcus-immune rabbits. Six rabbits (2 allergic, 2 pneumococcus-immunes and 2 normals) were injected subcutaneously with 1 cc. of a 5 per cent solution of egg albumin. Later (2 hours in one group and 3 hours in the other), 10^{-7} cc. of pneumococcic culture were injected through the original needle path into the field of developing inflammation. Cultural tests of this dilution yielded 56 colonies of pneumococcus. The results (Table III) show that

Table III

The Comparative Resistance of Allergic, Immune, and Normal Rabbits to Subcutaneous Injections of Virulent Pneumococci into a Developing Inflammatory Focus

Animal No.		Age of inflammation in hours	Dilution of pure culture of pneumococcus	Fate: hours until death
32	Egg albumin-allergic	2	10^{-7}	48
33	Pneumococcus-immune	2	10^{-7}	Lived
34	Normal	2	10^{-7}	72
35	Egg albumin-allergic	3	10^{-7}	72
36	Pneumococcus-immune	3	10^{-7}	Lived
37	Normal	3	10^{-7}	48

neither normergic nor allergic inflammation of from 1 to 3 hours duration prevented bacterial generalization and death. The pneumococcus-immune rabbits, however, showed no ill effects from the injection, demonstrating again the superiority of active specific immunity over non-specific inflammation as a protective mechanism.

Morphological Findings in Lesions of Normal, Allergic, Allergic-Immune, and Immune Rabbits Following the Subcutaneous Injection of Virulent Pneumococci

Finally, 13 rabbits were injected subcutaneously with the albumin-pneumococcus mixture in order to secure tissues for histological examination. Three animals allergic to egg albumin, 3 both

allergic to egg albumin and immune to pneumococcus, 4 immune to the pneumococcus alone, and 3 normals received dilutions of pneumococcic culture suspended in egg albumin ranging from 10^{-5} cc. to 10^{-8} cc. Cultures from 10^{-8} cc. yielded only 4 colonies of pneumococcus. The results are shown in Table IV. The comparison of the survival time in this series is not strictly accurate inasmuch as the survivors were sacrificed in order to obtain tissues comparable to those of the animals that died spontaneously.

TABLE IV

The Comparative Resistance of Normal, Egg Albumin Allergic, Pneumococcus-Immune, and Egg Albumin Allergic-Pneumococcus Immune Rabbits to Subcutaneous Injection of Virulent Pneumococci

Animal No.		Amount of culture in cc.	Fate of animals: hours until death	Remarks
38	Allergic	10^{-8}	48	
39	Allergic	10^{-8}	48	
40	Allergic-immune	10^{-8}	Lived	Killed for sections
41	Allergic-immune	10^{-8}	Lived	Killed for sections
42	Immune	10^{-8}	Lived	Killed for sections
43	Normal	10^{-8}	72	
44	Allergic	10^{-7}	48	
45	Allergic-immune	10^{-7}	Lived	Killed for sections
46	Immune	10^{-7}	Lived	Killed for sections
47	Normal	10^{-7}	48	
48	Immune	10^{-5}	Lived	Killed for sections
49	Immune	10^{-5}	Lived	Killed for sections
50	Normal	10^{-5}	48	

Nevertheless, there is the same general trend as in the three previous series. All of the animals immune to the pneumococcus and those both immune to pneumococcus and allergic to egg albumin were alive and healthy at the time when the normal rabbits and those allergic to egg albumin had died. It is obvious, again, that allergic inflammation failed to prevent a fatal outcome even when only a few pneumococci, probably not more than from 5 to 10 microorganisms, were injected.

The tissues were fixed in Zenker's solution with 5 per cent glacial acetic acid, were embedded in celloidin, and the sections were stained with hematoxylin and eosin, hematoxylin-eosin azure, and with the Gram stain by the Wallace modification.[6] The findings will be described briefly for each group.

Local Lesions in Normal Rabbits

The age of the lesions examined in 6 normal rabbits ranged from 18 to 40 hours, but all of them were essentially similar histologically and corresponded closely to the typical pneumococcic lesion of the rabbit as described by Rhoads and Goodner.[7] They were extremely edematous and the collagenic fibers were markedly separated. Leukocytes were numerous in some areas while almost absent in others, particularly toward the edge of the lesion (Fig. 1). Recent hemorrhage, threads of fibrin, and occasional thrombosed lymphatics were seen. The pneumococci appeared as small, compact and well stained diplococci and were uniformly distributed with no indication of clumping, colony formation or capsular swelling. Occasionally they were seen within leukocytes, but for the most part there was but slight phagocytosis. The microorganisms unquestionably had proliferated abundantly and had spread evenly through the edematous tissues with very little hindrance from the inflammatory reaction.

Lesions in Rabbits Allergic to Egg Albumin

The gross lesions at from 24 to 48 hours after infection were typical of the well developed Arthus reaction. Examination of sections showed massive edema, coagulation necrosis, recent hemorrhage, vascular thrombosis and an intense infiltration of leukocytes, particularly granulocytes. The pneumococci, however, were abundant, indicating that they had grown freely, and were evenly distributed throughout the area of inflammation (Fig. 2). They showed no tendency to adhere to one another or to grow into colonies. Phagocytosis was fairly active in some areas, but the leukocytes had obviously failed to prevent bacterial proliferation to any marked degree.

Lesions in Rabbits Both Allergic to Egg Albumin and Immune to Pneumococcus

The histological picture was essentially similar to that described for the allergic rabbits but the morphological characteristics of the pneumococci were quite different. The microorganisms were less numerous than in the normal or allergic animals, they were frequently swollen and their capsules were prominent, resembling

those seen in a positive Neufeld reaction (Fig. 3). In some instances isolated colonies of pneumococci were also present in acellular areas (Fig. 4). Small clumps of adherent swollen microorganisms indicated an agglutinative process. Phagocytosis was not particularly conspicuous at the borders of these areas.

Local Lesions in Rabbits Immune to Pneumococcus

Here there was but little evidence of inflammation and pneumococci were exceedingly difficult to find in the field of inflammation although they could be seen occasionally within leukocytes. The bacteria had evidently been engulfed so quickly after their entrance into the tissues that very little bacterial proliferation occurred; consequently, very little inflammation ensued (Fig. 5).

Discussion

An extensive discussion of these experiments is unnecessary inasmuch as we were not concerned with the general problem of allergy and its relation to resistance, but only with the ability of an allergic inflammatory reaction to restrict the growth and spread of virulent bacteria. Neither were we concerned with the relation of bacterial invasiveness to inflammation.[8] We wished only to ascertain whether a local allergic inflammation may hinder the escape of unquestionably virulent microorganisms from the region of developing infection. Our experiments indicate that any such hindrance is negligible.

The most conspicuous feature of our investigation was the uniformity and regularity with which both ordinary and allergic inflammation failed to prevent the escape of virulent pneumococci from their site of lodgement, both in non-inflamed tissues and in those undergoing early inflammation. Every normal and allergic animal died with no significant difference in the length of infection time. Histological examination of the local lesions in the allergic animals showed, furthermore, that conditions favorable for the local coagulation of plasma, thrombosis of lymphatics and accumulation of leukocytes failed either to prevent abundant proliferation of the bacteria or to modify their spread to the body as a whole. This is not surprising, however, as there is no reason to suppose that bacteria able to grow in the body fluids and within the leukocytes of a susceptible animal should be influenced adversely by a

greater accumulation of such fluids and cells. Vorwald,[9] in fact, has shown that if equal numbers of tubercle bacilli are injected into an animal of high resistance (cat) and one of low resistance (guinea pig), the intensity of the inflammatory reaction varies inversely with the degree of resistance rather than directly. Nor is it surprising that inflammation fails as a defensive mechanism under conditions in which bacteria can proliferate so abundantly in the inflammatory exudate itself.

The uniformity with which the pneumococci in the allergic lesions disseminated raises the question whether the edema and necrosis accompanying the inflammation did not favor bacterial growth and spread. Even in the rabbits both allergic to egg albumin and immune to the pneumococcus, bacterial growth was more abundant than in those only immune to the pneumococcus. Field, Drinker and White [10] have demonstrated the early increase in lymph flow from an area of inflammation, and Rhoads and Goodner [7] observed that pneumococci in the dermal lesions of rabbits reach the adjacent tissues by the edema fluid rather than because of their inherently invasive properties. Angevine [11, 12] showed that when hemolytic streptococci, either virulent or relatively avirulent, are injected intradermally into rabbits sensitized or immunized to them, they multiply more rapidly for several hours and persist longer in the local lesions than in the lesions of the corresponding controls. He concluded that "in sensitized animals local injury with necrosis favors the multiplication of relatively avirulent streptococci at the site of entry and explains their survival at a time when they have disappeared in the controls." Lurie [13] more recently has found that when he injected tubercle bacilli suspended in melted agar into normal and tuberculous rabbits, the microorganisms spread more rapidly to the regional lymph nodes of the tuberculous (allergic) than to those of the normal animals. It is evident from these experiments, as well as from our own, that rather than acting as an effective localizing agency, the allergic inflammation may actually increase the local vulnerability.

Another significant finding was the character and extent of the growth of the pneumococci in the different types of local lesions, particularly in the rabbits both allergic to egg albumin and immune to the pneumococcus. The presence of many large pneumococcic colonies as long as 40 hours after the injection of from approxi-

mately 5 to 50 pneumococci reemphasizes the fact that extracellular antibacterial substances exert but slight effect upon Gram-positive microorganisms.[14] Nevertheless, an extracellular antibody-effect exists, as evidenced by the enlarged diplococci with greatly swollen capsules (Fig. 3). This change is due, presumably, to the prolonged action of immune substances upon the bacterial surfaces, thus causing them to become sticky and to adhere to one another and to the tissues. The not infrequent occurrence of small clumps of these swollen bacteria indicates, furthermore, that increased cohesiveness has also initiated the process of agglutination in the tissues.

The insignificant local lesions in the rabbits immune to the pneumococcus, the histological evidence of only small foci of leukocytes in which pneumococci were almost absent, and an absence of any significant degree of fibrin deposition or thrombosis of lymphatics reinforces the general argument of this paper that inflammation, *per se*, plays no essentially determining part in the primary localization of virulent bacteria. It is altogether likely that when virulent bacteria enter immune tissues in the small numbers introduced in these experiments they are localized so sharply and engulfed so quickly that there is very little stimulus for the outpouring of any considerable amount of inflammatory exudate, so that at the end of from 36 to 40 hours the gross lesion is practically invisible and the microscopic findings are minimal.

Summary and Conclusions

Experiments were performed in an attempt to ascertain to what extent the accelerated and intense inflammatory reaction of allergy can act as a protective mechanism in a susceptible animal infected with a highly virulent microorganism. Fifty rabbits were used as follows: Some were sensitized to egg albumin by a series of subcutaneous injections until they became Arthus-positive; others were similarly prepared but were also immunized against a type I pneumococcus by injection of formol-killed cultures; a third group was immunized in the same way against the pneumococcus but was not sensitized to egg albumin, and a fourth group served as a control. The essential experiments consisted in mixing various dilutions of young broth cultures of the pneumococcus with egg albumin, injecting the mixture subcutaneously, and ob-

serving the development of the inflammatory lesion, the histopathological changes occurring therein, and the fate of the animal. The results may be summarized as follows:

1. As few as 4 pneumococci when suspended in 5 per cent egg albumin and injected subcutaneously caused the death within from 36 to 40 hours of all of the normal rabbits as well as those allergic to egg albumin. Allergic inflammation, therefore, failed to protect the rabbit against infection with virulent microorganisms. The pneumococci, furthermore, grew profusely in the areas of allergic inflammation, and spread evenly through the edematous field of inflammation. Phagocytosis, although moderately active, did not modify the course of the infection either in time or degree, and the pneumococci grew freely in the exudate itself. In other words, local conditions in the field of inflammation had no determining influence ultimately on the course of the infection.

2. The pneumococci also grew freely for a time in the lesions of rabbits both allergic to egg albumin and immune to the pneumococcus, but the microorganisms tended to remain localized near their sites of introduction. Here they developed into isolated colonies or became swollen, with prominent capsules, suggesting the appearance of a positive Neufeld reaction. Many of them lost their Gram-positive characteristics, presumably as a result of the extracellular action of antipneumococcic immune substances in the edema fluid. The animals, however, suffered no serious effects from the infection.

3. The lesions in actively immunized rabbits were small and inconspicuous after from 36 to 40 hours. Leukocytes were assembled in clusters in the area of infection but contained very few visible pneumococci, indicating the superiority of specific immunity over non-specific inflammation, whether normergic or allergic, in restricting the growth and spread of the microorganisms through the tissues.

4. These experiments, therefore, offer no support to the view that an inflammatory reaction can develop quickly enough to prevent the escape of virulent bacteria from their site of lodgement in previously non-inflamed tissue and cast further doubt on the validity of the hypothesis of a mechanical "walling off" as an important mechanism for bacterial fixation in a non-immune animal.

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DESCRIPTION OF PLATE

PLATE 19

FIG. 1. Area of subcutaneous inflammation in a normal rabbit injected with 10^{-7} cc. of pneumococcic culture. Death in 36 to 40 hours. Note the large numbers of pneumococci which have proliferated freely and have spread uniformly through the area of inflammation. Note also the absence of leukocytic infiltration. Gram stain (Wallace modification). × 2000.

FIG. 2. Area of subcutaneous inflammation in a rabbit allergic to egg albumin and injected with 10^{-8} cc. of pneumococcic culture (approximately 4 pneumococci). Death in 36 to 40 hours. Note the large numbers of pneumococci which have multiplied and spread diffusely despite a marked infiltration of leukocytes. Gram stain (Wallace modification). × 2000.

FIG. 3. A portion of the field of inflammation in a rabbit both allergic to egg albumin and immune to pneumococcus. Injected subcutaneously with a pneumococcus-egg albumin mixture containing 10^{-8} cc. of pneumococcic culture. Rabbit sacrificed in 42 hours. Note the greatly swollen pneumococci and the prominent capsules. Gram stain (Wallace modification). × 2000.

FIG. 4. Section of a subcutaneous lesion in a rabbit both allergic to egg albumin and immune to pneumococcus and injected with egg albumin-pneumococcus mixture containing 10^{-8} cc. of pneumococcic culture (approximately 4 microorganisms). Animal sacrificed in 42 hours. Note the colonies of pneumococci which have developed despite the fact that the animal had been specifically immunized. Gram stain (Wallace modification). × 310.

FIG. 5. Area of subcutaneous inflammation in a rabbit specifically immunized and injected with the egg albumin-pneumococcus mixture containing 10^{-5} cc. of pneumococcic culture (approximately 7000 pneumococci). Animal sacrificed in 44 hours. Note the large numbers of leukocytes and the almost complete absence of pneumococci. Gram stain (Wallace modification). × 2000.

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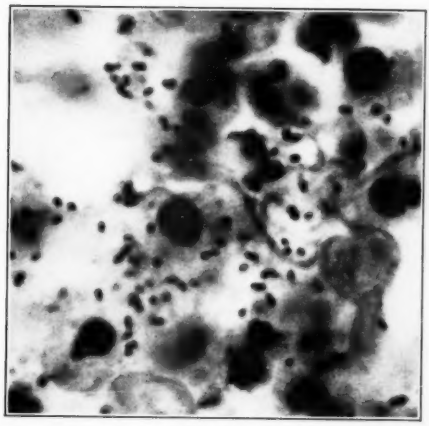

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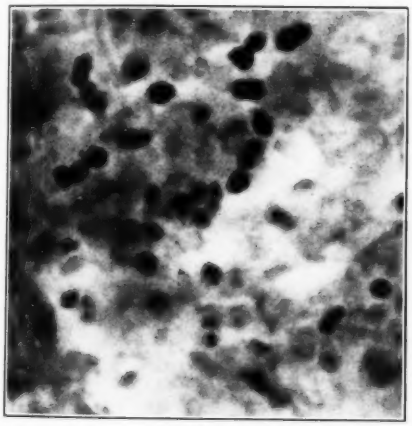

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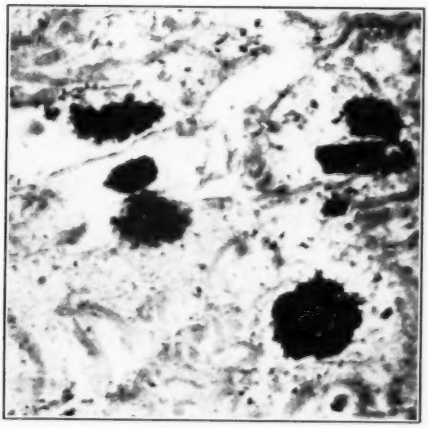

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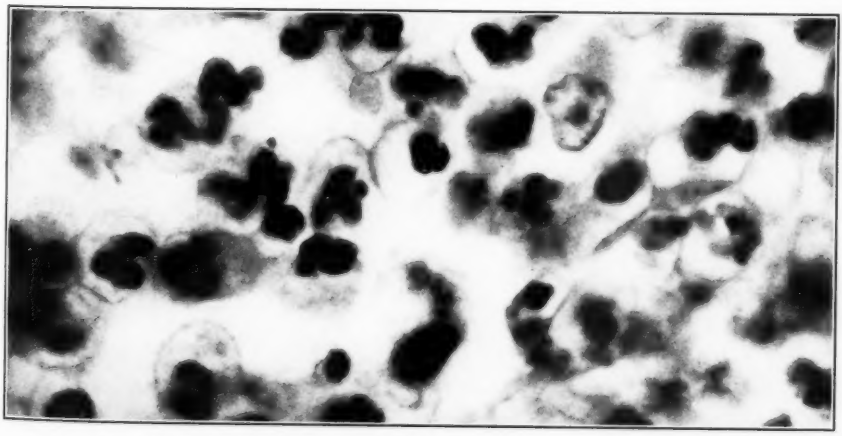

5

FATTY INFILTRATION AND CIRRHOSIS OF THE LIVER IN DEPANCREATIZED DOGS MAINTAINED WITH INSULIN *

I. L. CHAIKOFF, M.D., C. L. CONNOR, M.D., AND G. R. BISKIND, M.D.

(From the Division of Physiology (Berkeley), and the Division of Pathology (San Francisco), of the University of California Medical School)

It has been shown in this laboratory that completely depancreatized dogs treated with insulin survive for long periods when maintained on a diet adequate in calories, proteins, salts and vitamins, but lacking pancreas.[1] Under such conditions a number of pathological changes appear in the tissues of these animals. Bilateral cataracts have been found as early as 1 year following pancreatectomy.[2] A disturbance has also been found in the blood lipids: all constituents, in particular cholesterol esters, have markedly fallen soon after excision of the gland.[3] The most striking change occurs in the liver, in which large amounts of fat are deposited. These fat changes in blood and liver appear in the absence of pancreas in the diet, for by the addition of pancreas the fall in blood lipids, as well as the accumulation of fat in the liver, can be prevented.[4]

For the present report, the anatomical changes associated with fatty livers have been examined at various intervals following pancreatectomy. Although fatty livers may appear early and remain for long periods following excision of the pancreas, a regression in the fat content of the liver finally occurs. In 3 dogs that survived between 4 and 5.5 years the fat content of the liver returned to levels close to normal. During this entire period of observation the livers of the 49 dogs examined showed two types of lesions. The first of these occurred in association with the early infiltration of fat, while the second or final stage appeared most characteristically in those livers in which the regression of fat had taken place. Such livers showed an extensive periportal fibrosis with irregular lobulation indicative of cirrhosis. The depancreatized dog thus provides a new method for the production of

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experimental cirrhosis, this occurring as a final stage in response to a fatty infiltration of long standing in the liver.

Experimental

The preparation and treatment of the depancreatized dogs employed in this study have been described elsewhere.[1] After pancreatectomy each dog received twice daily a mixture containing meat, sucrose and bone ash. Vitamin supplements (A and D as cod liver oil *; the B complex in the form of a concentrate obtained from rice bran **) were added to the diet mixture twice a week. Each dog received 16 units of insulin daily, 8 units at each time of feeding.

A careful search for pancreatic tissue was made in all depancreatized dogs at autopsy. The completeness of pancreatectomy in all animals recorded in this study was verified.

The autopsies were performed immediately after the animals were sacrificed or within a few hours after death. A complete autopsy was performed in every case, and blocks of tissue were removed from all organs for histological study. After the blocks were removed from the liver for histological examination, the rest of the organ was immediately ground and thoroughly mixed, and samples were taken for determinations of total fatty acids, cholesterol and phospholipids. The methods employed for lipid analyses have been described elsewhere.[4]

I. The Fatty Changes in the Liver after Pancreatectomy

Although, as already pointed out, large amounts of fat may appear in the liver soon after pancreatectomy, extremely fatty livers are not of constant occurrence in the early periods. Not only the degree of lipid infiltration, but also the time of onset of such changes show considerable variation. Thus, mixed samples of the entire livers obtained from 2 dogs that received the same treatment contained 32 and 11 per cent of fatty acids at an interval of 3.5 weeks after pancreatectomy. Equally significant variations were found at other intervals. In a series of 30 dogs, however, it

* The standardized cod liver oil used in this study was kindly furnished in part by Mead Johnson and Company, Evansville, Indiana.

** The rice bran concentrate was kindly furnished by Vitab Products, Inc., Emeryville, California.

was found that fatty acids in excess of 14 per cent were present at an interval of 20 to 30 weeks after excision of the gland.

In the normal dog's liver the lobules are not outlined by limiting strands of connective tissue and there is an extremely scanty amount of connective tissue associated with the portal triads. The finer biliary radicles are usually difficult to demonstrate. The central veins can be distinguished: they are situated at regular distances from the portal triads. The cords of hepatic cells are evenly arranged, and the sinusoids radiate uniformly from the portal triads to the central veins. The hepatic cells occasionally contain a small vacuole which is shown by a scharlach R stain to be fat. By chemical analyses such livers have been shown to contain approximately 3 to 4 per cent of fatty acids.

Microscopic Appearance of Livers Containing Various Amounts of Fat Obtained from Depancreatized Dogs in Good Condition

The livers were removed at various intervals after pancreatectomy. In livers containing relatively small amounts of lipids (about 10 per cent), the fat was deposited in large globules replacing the cytoplasm of cells in scattered areas within a lobule (Fig. 1). In some cases the fat globules were irregularly distributed toward the mid portion of a lobule. No change other than the usual displacement of the nucleus was observed in the cells of these livers. In general, the size of the fat globules was uniform.

In livers showing a greater lipid change (approximately 18 per cent), fat was found in almost every cell of the lobule (Fig. 2). The largest globules of fat were generally found in the cells around the centers of the lobules, while the remainder of the cells showed small droplets scattered throughout the cytoplasm. In many of the cells around the portal triads the cytoplasm had become granular and somewhat hyaline in appearance. There were no nuclear changes. The sinusoids were not easily distinguished. The cells of the large bile ducts occasionally contained fat in their cytoplasm.

When about 30 per cent of fat had accumulated in the liver, almost every hepatic cell was completely replaced by fat (Fig. 3). A rare cell, however, retained some cytoplasm which frequently had a hyaline, granular appearance. The sinusoids were completely obliterated and their compressed cells varied in size and

shape. Nuclei may not be present in every cell. There were no changes in the structure of the portal triads. Nearly all the lining epithelial cells of the bile ducts contained fat.

Distribution of Fat in the Liver

The distribution of lipids was studied in the livers of depancreatized dogs in which various degrees of fat infiltration were produced experimentally. Blocks of tissue, varying from 4 to 6 sq. cm. in area and from 3 to 4 mm. in thickness, were removed from each of the lobes for histological examination. Three consecutive slices were removed from each block of tissue. The middle section was used for histological study while the two end slices were combined for chemical analysis.

In livers containing little fat this was distributed in a fairly regular and equal manner throughout the various lobes. Moreover, the sections showed that the fat was evenly distributed within the lobules (Fig. 1). With increasing amounts of fat, however, the various lobes at times showed extreme variations in their fat content (Fig. 2). This was confirmed by chemical analyses. The lobular distribution of lipids likewise varied in these cases; some showed nearly all of the cells filled with fat-stained globules, while others had fat only in the peripheral cells or around the central vein. In a liver containing about 50 per cent of fat the lobes showed very little variation in lipid distribution and the lobules showed all cells uniformly filled with fat (Fig. 3). Thus, while livers that contain either very small or very large amounts of lipids have the fat uniformly distributed, livers in which a moderate degree of lipid infiltration has occurred may show an uneven distribution of fat. The storage and liberation of fat apparently do not occur in a constant or regular manner in the various parts of the liver.

II. Early Fibrotic Changes

A second group of livers developed, in addition to the fatty changes noted above, a prominence of the portal spaces caused by a fibrous tissue proliferation that is never seen in the normal liver. The lobulation, however, is not complete, nor is the extensive distortion of the parenchyma so marked as in the cirrhotic livers to be described below. There is some proliferation of the bile ducts. The hepatic cells near the portal fibrous tissue septums generally

show a hyaline granular alteration of the cytoplasm, and occasional cells have undergone marked fatty changes. All these livers show a variable degree of lymphocytic and plasma cell infiltration in association with the growing connective tissue.

1. Dog G8: Female, depancreatized April 2, 1931. This animal survived for 3.3 years after pancreatectomy, at the end of which time it was sacrificed for examination of the liver.

Microscopic Appearance of Liver: The portal spaces were prominent, as there was definite fibroblastic proliferation around them, which gave the liver a definite lobular pattern. The hepatic cells showed extreme variation in fat content, large groups of cells being completely filled whereas adjacent groups had a granular hyaline cytoplasm with no visible fat. The central veins were not easily distinguished (Fig. 4).

2. Dog DJ: Female, depancreatized June 29, 1932. This animal was in good condition until 3 days before Feb. 4, 1934, when it died. The period of survival was 1.6 years. At autopsy an extensive retroperitoneal hemorrhage and cellulitis, which also involved the heart and aorta, were found. Focal hemorrhage and leukocytic infiltration of the right auricular muscle were present.

Microscopic Appearance of Liver: The histological appearance of this liver was similar in all details to that of Dog G8. The terminal acute infectious process had not altered the previous changes in the liver.

3. Dog DG: Female, depancreatized Aug. 1, 1932, died Feb. 14, 1934. Period of survival 1.5 years. This animal refused food for 2 weeks before death occurred. Autopsy revealed an acute urinary tract infection. A mixed sample of the liver contained 29.2 per cent of total lipids.

Microscopic Appearance of Liver: There was a diffuse fatty infiltration in all parts of the liver. The periportal fibrous tissue was arranged in rather thin bands and appeared condensed. Occasional leukocytes, lymphocytes and plasma cells were present in the fibrous tissue. Groups of cells lying against the fibrous tissue septums showed hyaline changes.

4. Dog K: Male, depancreatized Dec. 25, 1930, died Sept. 12, 1933. Period of survival 2.7 years. For several weeks before death this animal lost its appetite and finally refused all food. It was

emaciated at the time of death. At autopsy bronchopneumonia and an acute pyelitis were found. The liver weighed 920 gm. (*i.e.*, 14.2 per cent of the final body weight). A mixed sample of the whole liver contained 33.8 per cent of total lipids.

Microscopic Appearance of Liver: The tendency toward the formation of lobules by a slight periportal fibrosis was seen in all sections. All hepatic cells were well filled with fat, so that the peripherally situated cells rarely showed the hyaline alteration of the cytoplasm. Proliferation of the bile ducts was not distinct. The bile canaliculi occasionally contained plugs of inspissated bile. In the periportal fibrous tissue lymphocytes and plasma cells were rare.

III. Cirrhosis of the Liver in Depancreatized Dogs

Extensive cirrhosis was found in 4 dogs. In 2 of these (dogs DA and DB) the livers presented the characteristic hob-nailed appearance. The surface was reddish brown in color and was covered by nodules of varying size, the largest of which measured 10 mm. in diameter. The liver on cut section felt sclerotic, and the cut surface showed the parenchyma to be composed of irregular lobules. In the remainder of the animals the surfaces of the livers appeared normal. All livers were enlarged. In all cases the gall-bladder and the extrahepatic bile ducts were normal on gross examination. Bile could be expressed through the papilla by pressure on the gall-bladder. The animals were not jaundiced. All the organs were examined at autopsy and routine sections taken from each one. The protocols of these animals are as follows:

1. Dog DA: Female, depancreatized March 11, 1931. This animal was in good condition at the end of the period of maintenance, Sept. 25, 1936. Its period of survival was 5.5 years. At autopsy the liver was found greatly enlarged and hob-nailed in appearance. It weighed 565 gm., or 7.5 per cent of the body weight. A mixed sample of the liver contained 5.6 per cent of total lipids.

Microscopic Appearance of Liver: A striking amount of fibrous tissue was found around the portal spaces (Fig. 5). These spaces were irregularly distributed, and the fibrous tissue branched from one to another. In this manner the liver parenchyma had been divided into sharply circumscribed lobules that varied extremely in size and shape. Glisson's capsule was greatly thickened. The

central veins were not easily distinguishable and were eccentrically placed. Within the lobules the hepatic cells were arranged in distorted cords compressing the sinusoids. These cells contained a variable amount of fat; in some lobules many cells might contain a large globule of fat, whereas in the adjacent lobule the cells might have very little. Some cells had a markedly vacuolated cytoplasm, whereas others contained a cytoplasm of a granular or hyaline nature. There was a great variation in the thickness of the fibrous septums. Usually there was an associated lymphocytic and plasma cell infiltration, together with a prominent proliferation of the small bile ducts. In the fibrous septums a group of hepatic cells would often be enclosed, and these cells generally showed a condensation of the cytoplasm into hyaline granular masses (Fig. 6).

2. Dog DB: Male, depancreatized July 27, 1932. This animal was in good condition at the end of the period of maintenance, Sept. 25, 1936. Its period of survival after pancreatectomy was 4.2 years. At autopsy the liver was found greatly enlarged and its surface hob-nailed in appearance. It weighed 400 gm., or 5.7 per cent of the body weight. A mixed sample of the entire liver contained 3.5 per cent of total lipids.

Microscopic Appearance of Liver: This was similar in all details to that observed in Dog DA described above.

3. Dog DC: Female, depancreatized Sept. 1, 1931. This animal was in good condition at the end of the period of maintenance, Sept. 29, 1936. Its period of survival was 5.1 years. At autopsy the liver was found enlarged, weighing 480 gm., or 4.5 per cent of the total body weight. A mixed sample of the whole liver contained 3.9 per cent of total lipids.

Microscopic Appearance of Liver: Connective tissue was present in fine radiating strands usually extending outwards from the portal triads in irregular fashion. The lobular pattern could be readily distinguished, but the fibrous tissue septums were not so prominent as in dogs DA and DB. The hyaline alteration of the cytoplasm of the cells at the periphery of the lobule was, however, more striking in this animal than in those described above. Some cells were also undergoing marked shrinkage or showed complete replacement of the cytoplasm by fat.

4. Dog DE: Depancreatized Sept. 5, 1932. This dog was maintained for 2.3 years on the stock diet recorded above, which contained no raw pancreas. For the next 15 weeks it received 250 gm. of raw pancreas daily in addition to the regular stock diet, and at the end of this time it was sacrificed for study of the liver. The total period of survival was 2.6 years, during which the animal was in good condition. The liver was large and weighed 640 gm., or 7.4 per cent of the body weight. A mixed sample of the entire liver contained 4.6 per cent of total lipids.

Microscopic Appearance of Liver: The histological structure was similar to that observed in Dog DC.

In 6 dogs that survived for periods longer than 1 year no cirrhosis or abnormal degree of fibrosis was found in the liver. One of these (Dog DF) was examined 3.1 years after removal of the pancreas, whereas in the 5 other animals (dogs G1, A1, A_3, G_3 and G_2) the livers were removed for study at intervals between 1.3 and 1.8 years after pancreatectomy.

Discussion

The results of the present investigation demonstrate the occurrence of cirrhosis of the liver under conditions not hitherto described. Sixteen completely depancreatized dogs were maintained for periods longer than 1 year. The tissues in 14 of these animals were subjected to a careful histological study. Extensive cirrhosis was found in 4 dogs, while in 4 others an abnormal degree of fibrosis was present. Infection seems to play no part in the production of this scarring, for the 4 dogs in which the most marked cirrhosis was found showed no other pathological changes when sacrificed. Incidental acute infections were present in 3 of the second group of 4 dogs. From the appearance of the livers, however, it is obvious that these terminal infections are in no way related to the fibrotic changes that occurred. In all dogs recorded in this study there was no evidence of obstruction in the extrahepatic bile passages at autopsy. The type of lesion produced does not resemble in any particular the changes associated with infection or extrahepatic biliary obstruction.

A constant finding in all these dogs is an early increase in the amount of fat in the liver. This usually takes place in the first few

months after pancreatectomy and remains for long periods. It was shown by chemical analyses that a fatty liver may be present as late as 3 years after pancreatectomy. The fat first appears in scattered cells within a lobule and slowly extends outward from the central veins. The peripheral cells, however, are the first to show cytoplasmic alterations and this is followed by hyaline atrophy of the whole. The process is apparently slow and stimulates avascular fibroblastic proliferation. A few lymphocytes and plasma cells accompany this reaction. Strands of fibrous tissue then intertwine around other peripherally located cells, thus isolating them and making the process a progressive one.

In all livers showing fibrosis some cells can always be found which show extreme hyalinization and granularity of the cytoplasm. These granules may be pushed off to one side if a fat globule is present in the cell. Usually the cells containing this marked cytoplasmic change are arranged in small groups.

The time of onset of these fibrous changes in the liver remains to be considered. No direct relation between the interval after pancreatectomy and the degree of fibrous proliferation was found in the 8 dogs studied. Thus, while all the animals that survived between 4.2 and 5.5 years showed marked cirrhosis, a greater degree of fibrosis was found in 1 dog 2.6 years after pancreatectomy than in another 3.3 years after. No evidence of fibrosis was found in the first few months after pancreatectomy; the earliest signs of such changes occurred after an interval of 1.5 years.

Summary and Conclusions

Depancreatized dogs constantly develop fatty livers at variable periods after the operation has been performed. In those kept for from 2.6 to 5.5 years upon an adequate diet and insulin, 8 of 16 developed more or less interlobular fibrosis of the liver associated with a hyaline or colloid degeneration of many cells and hyaline atrophy of peripheral cells. In 4 animals this was so pronounced, both grossly and microscopically, that the picture of a well advanced portal cirrhosis of the liver was present. By the time this severe cirrhosis had occurred, the fat content of the livers had returned to normal and there was little histological evidence that a markedly fatty liver had preceded the fibrosis. The sequence of events appears to be fatty infiltration, hyaline degeneration and

atrophy of cells at the periphery of lobules, and fibroblastic proliferation in orderly fashion, ending with the typical hob-nail appearance and fibrotic structure of cirrhosis. Necrotizing agents introduced from the outside, infection, and extra-hepatic biliary obstruction were excluded as causative factors.

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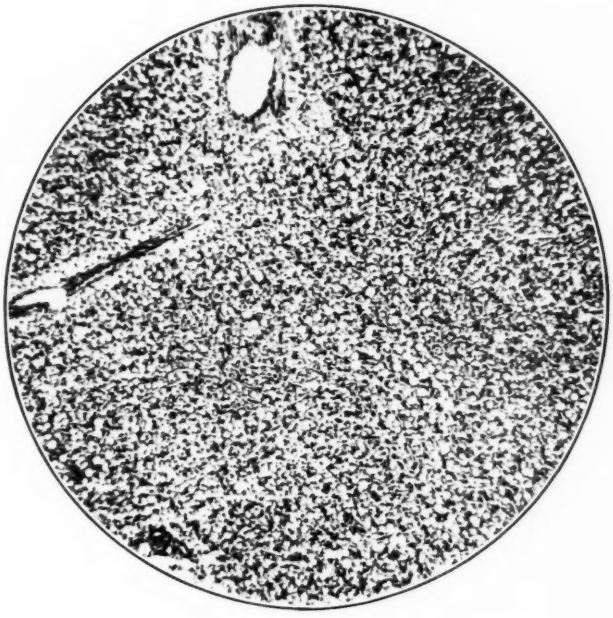
DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Liver of Dog D100A showing early stage of fatty infiltration 3.5 weeks after pancreatectomy. Fatty acid content 10.5 per cent. Hematoxylin-eosin stain. × 73.

FIG. 2. Liver of Dog D94D showing fatty infiltration of intermediate degree in liver 14.5 weeks after pancreatectomy. The liver immediately adjacent to this area contained 17.5 per cent fatty acids. Hematoxylin-eosin stain. × 73.



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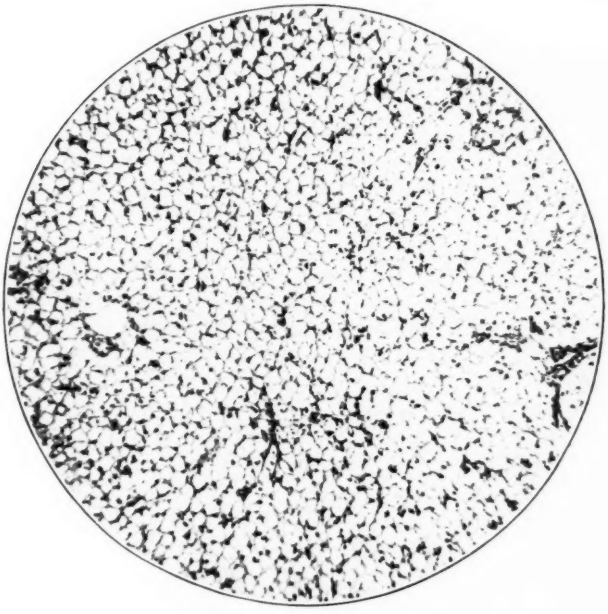
2

Chaikoff, Connor and Biskind Fatty Infiltration and Cirrhosis of Liver

PLATE 21

FIG. 3. Liver of Dog D1 showing advanced degree of fatty infiltration 34 weeks after pancreatectomy. A mixed sample of the remaining liver contained 26.3 per cent fatty acids. Hematoxylin-eosin stain. × 73.

FIG. 4. Liver of Dog G8 showing fibroblastic proliferation with decrease in amount of fat present 3.3 years after pancreatectomy. Phosphotungstic acid hematoxylin stain. × 73.



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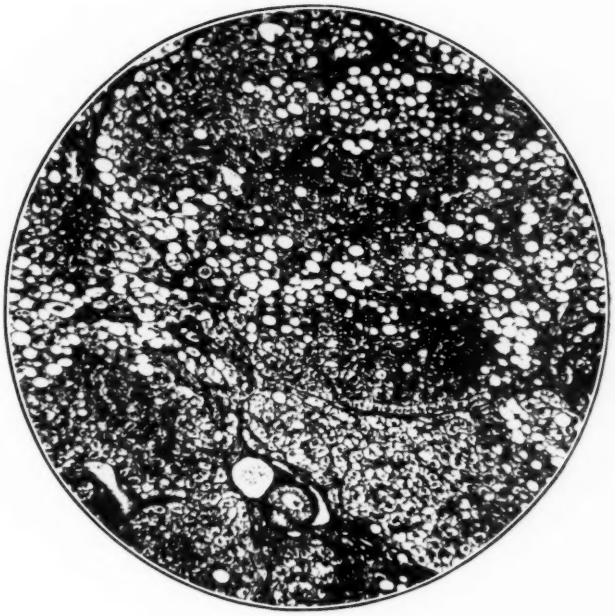


4

Chaikoff, Connor and Biskind Fatty Infiltration and Cirrhosis of Liver

PLATE 22

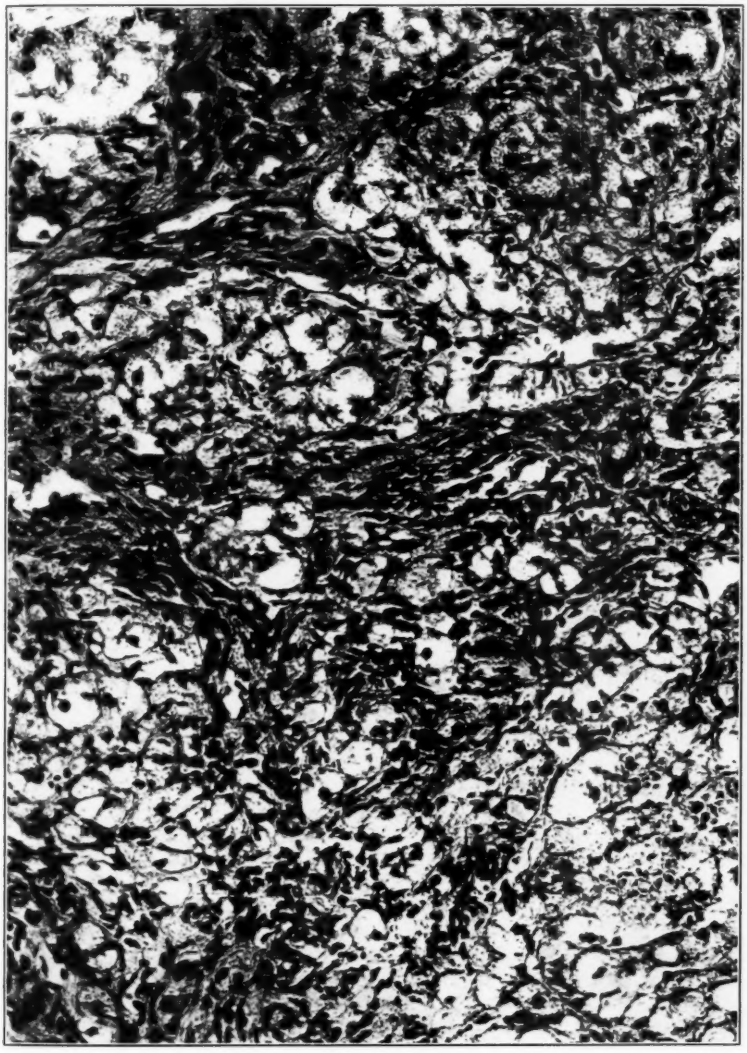
FIG. 5. Liver of Dog DA showing well advanced cirrhosis of the liver 5.5 years after pancreatectomy. There is very little fat present now. Total lipids 5.6 per cent. Phosphotungstic acid hematoxylin stain. × 38.

5

Chaikoff, Connor and Biskind Fatty Infiltration and Cirrhosis of Liver

PLATE 23

FIG. 6. Liver of Dog DA showing details of fibroblastic proliferation in liver shown also in Fig. 5. Fat not prominent in cells, but the cytoplasm in many cells is lumpy and hyaline in appearance. Some rounded masses resemble "colloid" bodies. Dark, irregular homogeneous masses between strands of connective tissue are atrophied hyaline liver cells. Hematoxylin-eosin stain. × 300.



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Chaikoff, Connor and Biskind Fatty Infiltration and Cirrhosis of Liver

PATHOLOGICAL CHANGES IN THE PLACENTA ASSOCIATED WITH ERYTHROBLASTOSIS OF THE FETUS *

LOUIS M. HELLMAN, M.D., AND ARTHUR T. HERTIG, M.D.

(From the Department of Pathology and the Department of Obstetrics, Harvard University Medical School and the Boston Lying-in Hospital, Boston, Mass.)

One of the earliest writers to recognize the importance of congenital edema of the fetus was Ballantyne.[1] He collected 70 cases, including those that had appeared in the literature and those that had come to his own personal notice. While the edema noted in many of these infants seemed to be due to obvious congenital defects, there were a few cases that showed no obvious etiological factor. It is this latter group that we have classified as part of the general syndrome of erythroblastosis, and it is in this group also that first mention is made of the gross placental changes in fetal dropsy.

Schridde,[2] first recognizing the underlying pathological changes associated with congenital hydrops, merely mentioned the placentas as showing edema both grossly and microscopically. In the same year Sitzenfrey [3] noted both edema and hyperplasia of the stroma of the villi. Nyhoff [4] stated that the villi were necrotic. Esch,[5] Eichelbaum,[6] Weiner,[7] and Kovács [8] also noted edematous changes in the villi. Goormaghtigh [9] felt that there was erythroblastic proliferation and infiltration of the stroma.

Diamond, Blackfan, and Baty [10] in an extremely comprehensive article on erythroblastosis have given a complete survey of the literature. In many of their references the authors have taken passing notice of the placental changes. This is especially true where the striking gross changes associated with congenital hydrops occur. In the same paper there appears a short microscopic survey of the pathological changes in the placenta.** For the first time mention is made of the apparent immaturity of the placenta. This feature has been similarly emphasized by Clifford and Hertig.[11]

At no time, however, has a systematic examination of the placentas associated with both congenital hydrops and icterus gravis been recorded. It is the object of this investigation to record the histopathological changes in the placentas of infants

* Received for publication July 30, 1937.

** Description of the placenta contributed by Hertig.

suffering from erythroblastosis. For this purpose erythroblastosis has been divided into two subgroups — congenital hydrops and icterus gravis. The diagnosis of erythroblastosis has been made on finding, either clinically or at postmortem examination, an enlarged liver and spleen associated with an abnormal number of circulating nucleated red cells and extramedullary erythropoiesis. The term congenital hydrops has been used to designate the additional finding of anasarca, with or without icterus. The infants showing the additional finding of icterus without anasarca were classified under the term icterus gravis (Hellman and Hertig [12]). The majority of the cases herein presented occurred in the Boston Lying-in Hospital between the years 1931 and 1937. A few cases, however, were submitted to the pathological laboratory of this hospital for confirmation of diagnosis. These have been included with the permission of the attending physicians. The mothers of all the infants had negative blood tests for syphilis.* In addition, the liver, spleen and placenta of all infants in the hydrops group were stained for spirochetes by the Levaditi method. In no instance were any spirochetes found. For the sake of brevity only 1 case in each of the two groups will be presented in detail. The remainder will be presented in tabular form.

Presentation of Cases

Type 1. Congenital Hydrops

Case 1. Mrs. H. B., No. 71756. The mother was a 29 year old, white, American-born para 5. She had had 2 normal children, 2 miscarriages and 1 stillbirth. Her prenatal course during this pregnancy was normal until 2 weeks prior to admission to the hospital when she began to have headaches. She developed a rapidly progressive type of toxemia and was admitted to the hospital as an emergency case. On admission she had edema of the hands, face and extremities, a blood pressure of 150/90, and a large trace of albumin in the urine. The laboratory test for syphilis was negative. In spite of all therapy she became progressively worse. On March 24, 1937, when she was 8 weeks from term, a Braxton Hicks version was performed and a foot brought down. She was subsequently delivered of a 2880 gm. stillborn female infant with advanced generalized edema. The clinical diagnosis of erythroblastosis of the hydrops variety was confirmed at postmortem examination.

Description of Placenta

The placenta weighed 1260 gm. The membranes were complete but badly lacerated. The fetal surface was smooth, shiny,

* The Hinton test for syphilis was used throughout.

gray in color and translucent. The cord showed no gross pathological change other than edema. The maternal surface was intact, a pale yellow gray, deeply fissured and extremely friable (Fig. 1). There was no calcification of the decidua. The placenta cut with increased resistance and the cut surface was a pale yellow gray and firm in consistence. The villi could be easily teased out.

On microscopic section the villi were larger than normal. The syncytial cells were large, and the nuclei were regularly spaced, large and vesicular. There were frequent paranuclear clear spaces which had a tendency to depress the adjacent nuclei. These vacuoles contained neither stainable fat nor glycogen. There were few so-called nuclear knots. There was a partial persistence of Langhans' layer. The villi presented stroma of both the hyperplastic and the edematous types, but the former was by far the more common. The hyperplastic type of stroma was made up of small cells with well stained acidophilic cytoplasm and small dark nuclei. The edematous type of stroma had fewer cells and these were widely separated by clear spaces. These cells were large, had a basophilic or pale acidophilic cytoplasm, were multipolar and gave off long fibrils. The nuclei were large and vesicular with peripherally arranged chromatin. In the interstices were many large mononuclear cells with a granular acidophilic cytoplasm (Hofbauer cells) which was often vacuolated. These vacuoles contained fat. The vessels were diminished in number and had a tendency to be arranged peripherally. The endothelium of the vessels was made up of large cells whose nuclei projected into the lumens of the vessels. The capillaries and larger vessels were all filled with nucleated red cells. In the villi with hyperplastic stroma were many foci of intracapillary erythropoiesis which showed red cells in all stages of development (Fig. 4). There were a few areas of subsyncytial fibrinoid deposition. The decidual and chorionic plates showed no pathological changes.

Type 2. Icterus Gravis

CASE 2. Mrs. S. L., No. R.H. 2673. The mother was a 38 year old, white, American-born para 2. Her first child was normal. The prenatal course during this pregnancy was entirely normal. The laboratory test for syphilis, although not done during the prenatal period, was negative on a follow up visit. She was delivered normally at term on Dec. 10, 1932, of a 3360 gm. male infant. The child was covered with a golden yellow vernix and was quite cyanotic at

TABLE I

Congenital Hydrops

Case No.	Age	Parity	Weight of placenta	Weight of fetus	Size of villi	Syncytial degeneration	Persistence of Langhans' layer	Epithelial vacuolization	Hyperplastic stroma	Edematous stroma	Diminution of vessels	Immature endothelium	Nucleated red cells	Erythropoiesis	Calcification	Infarction
	yrs.		*gm.*	*gm.*												
S.C. R.H.864	36	4	1230	4140	++	+	++	+	+++	+	++	+	++			
M.K. 2827	37	2	1160	2880	++	+	++	++	+++	++	++	+++	+++	+++		
O.B. R.H.2420	22	2	1150	3240	++	+	++	++	+++	+	+	++	+++	++		
A.G. 786	33	9	1140	2910	+++	++	++	++	+	+++	++	++	+++	++		
E.R. R.H.2616	32	4	1040	3840	+++	+	++	++	+++	++	++	++	+++	++		
R.C. 5279	40	11	800	2490	++	+	++	+	+++	++	++	++	+++	++		1
E.M. 2832	30	4	1220	3890	++	+	++	++	++	++	+	+	+++	++		
M.D. R.H.2777	25	2	865	3420	++	+	++	+	+	+		+	++			
E.C. 12938	37	4	990	2730	++	+	++	++	++	+	++	++	+++	++		

E.C. 13627	37	7	1275	3450	++	+	++	+	+++	+	++	++	++			
F.K. 20651	27	3	1790	2355	+++	+	++	+	+++	+	++	++	+++	++		
H.B. 71756	29	5	1260	2880	+++	+	++	++	+++	+	++	++	+++	++		
H.W. 14577	39	12	1300	3600	+++	+	++	++	+++	++	++	+++	+++	+++		
G.B. 19067	23	3	1080	2160	+++	+	++	++	+++	++	++	++	++	++	+	1
M.*	27	3	1183	3750	+++	+	++	++	+++	++	++	++	++	++		
W.†	29	4			+++	+	++	++	+++	++	++	++	++	++		

*Case submitted by Dr. A. E. Steele, Lawrence Memorial Hospital, Medford, Mass.
†Case submitted by Dr. R. Durkee, Hartford Hospital, Hartford, Conn.

TABLE II

Icterus Gravis

Case No.	Age	Parity	Weight of placenta	Weight of fetus	Size of villi	Syncytial degeneration	Persistence of Langhans' layer	Epithelial vacuolization	Hyperplastic stroma	Edematous stroma	Diminution of vessels	Immature endothelium	Nucleated red cells	Erythropoiesis	Calcification	Infarction
	yrs.		*gm.*	*gm.*												
E.N. 5435	21	2	810	3090		+++							+			
S.L. R.H.2637	38	2	570	3360	+	+	++	++	++	+	+	+	++			
C.S. 11649	27	2	610	3680	+	+++							+			
D.B. 16449	39	5	650	3360	+	+		+	+		+	+	+			
G.F. 16827	28	3	460	3210	+	++		++	+	+	+	+	+			
A.F. 8844	23	3	735	3750	+	++		+	+			+	+			
E.N. 5435	27	4	540	3570	+	+		+	+			+	+			

birth. He never breathed well. Shortly after birth he developed petechiae in the scalp. The liver and spleen were enlarged to palpation. Examination of the blood showed 4,100,000 red cells and 14,000 nucleated cells, 64 per cent of which were red cells. The hemoglobin was 105 per cent. The clotting time was 30 minutes, the bleeding time normal. In spite of a transfusion of 30 cc. of mother's blood the child continued to fail. The nucleated red cell count rose to 80 per cent of the nucleated cells. Jaundice appeared on the 2nd day and the child died shortly thereafter.

Permission for a postmortem examination was not obtained.

Description of Placenta

The placenta weighed 570 gm. The membranes were complete but were stained a slight yellow. The chorionic plate was thin, smooth and glistening. The maternal surface was intact, showed normal fissuring, and there was no calcification. There were several small areas of ischemic necrosis of the villi. The placenta cut with the usual resistance and the cut surface was pale reddish yellow. It was not unusually friable. The cord showed no pathological changes.

On microscopic section the villi were slightly increased in size. The syncytial cells were slightly larger than usual and the nuclei were more evenly spaced. The nuclei were large and vesicular and there were many paranuclear clear spaces that had a tendency to depress the adjacent nuclei. The so-called nuclear knots were somewhat reduced in prominence. There was a partial retention of Langhans' layer. The epithelial layers showed little degenerative change. The cellularity of the stroma was increased but was mostly of the edematous variety and the hyperplastic type was not as evident as in the placenta of Type 1. The same large cells with multipolar processes were present. In the interstices were many Hofbauer cells, most of which showed cytoplasmic vacuoles. The vessels were somewhat reduced in number but their endothelium showed no pathological changes. There were no areas of erythropoiesis but all the vessels contained nucleated red cells. The chorionic and decidual cells showed no pathological changes.

Discussion

Table I records the gross and microscopic pathological changes in 16 placentas from infants suffering from erythroblastosis of the hydropic variety. The infant mortality in this group was 100 per

cent and the diagnosis in each instance was confirmed by post-mortem examination. In considering these pathological changes only the outstanding deviations from normal have been recorded. In an effort to give some estimate of the magnitude of this deviation, the symbols +, ++, and +++ have been used. A blank space represents no deviation from the normal. These values were based on the opinion of one individual and in spite of the necessarily attendant error showed a remarkable consistency.

The pathological changes tabulated in Table I are also shown in the microphotographs (Figs. 2–5). The placentas were nearly double the normal weight and the fetal placental ratio was reduced from the normal of 6:1 to 3:1. Grossly the placentas presented a friable gray maternal surface which was quite distinctive. Microscopically the villi were increased in size, epithelial degeneration was reduced to a minimum, and there was an abnormal persistence of Langhans' layer, which should have entirely disappeared by the 5th month. Vacuolization of the epithelium was present in each instance. Definite changes in the stroma were also noted. There was a diminution in the number of vessels and the endothelial cells were large and immature. Nucleated red cells were always present and foci of erythropoiesis were noted in all but 3 of the placentas.

The 7 placentas recorded in Table II were from infants suffering from erythroblastosis of the icterus gravis variety. The presence of the disease in these infants was proved either at autopsy or by the clinical findings of an enlarged liver and spleen, jaundice, and a greatly increased number of circulating nucleated red cells. The placentas in this group were normal in weight but, as can be seen in Table II, they presented microscopic pathological changes varying only in degree from those seen in the preceding group.

It is felt that the changes here described constitute a pathological syndrome pathognomonic of erythroblastosis. The cause of these pathological changes is obscure. However, it is worth noting that the immature placenta, especially during the period of its greatest growth, shows epithelial, stromal and vascular changes reminiscent of those described above. However, erythropoiesis and nucleated red cells are never seen in the abundance in which they occur in erythroblastosis. It is of interest, too, that anaplastic proliferating epithelium from hydatidiform moles and cases of chorionepithelioma shows vacuolization similar to that seen in the

immature placenta and the erythroblastic placenta. The presence of the Hofbauer cells in the immature placenta and that of erythroblastosis is of undetermined significance.

The resemblance of these pathological changes to those described as occurring in the syphilitic placenta is striking. Grossly the placentas are similar except for the peculiar fatty surface attributed to the syphilitic placentas. Microscopically the villi in both types are enlarged. Here, however, the resemblance ends, for the pathological changes described as being due to erythroblastosis do not resemble the fibrosis, obliteration and round cell infiltration of villous vessels attributed to syphilis. Although syphilis probably does produce certain changes in the placenta, it is the consensus of current opinion that too often the presence of a positive maternal Wassermann has led to a diagnosis of syphilis of the placenta when the changes present were due primarily to immaturity. A more careful consideration of the autopsy and placental material in these cases would probably disclose a number of cases of erythroblastosis. It is certain that the postmortem examination of every stillborn infant should include both gross and microscopic examination of the placenta.

Summary and Conclusions

1. Sixteen placentas from infants suffering from erythroblastosis of the hydropic variety and 7 from infants suffering from erythroblastosis of the icterus gravis variety are presented.

2. Pathological changes in the epithelium, stroma and vascular tree are described which constitute a pathological syndrome pathognomonic of erythroblastosis of the hydropic variety.

3. Similar, but less advanced pathological changes are present in placentas from infants suffering from erythroblastosis of the icterus gravis variety.

4. These pathological changes resemble to some extent those seen in immature placentas and in the epithelium from hydatidiform moles and cases of chorionepithelioma.

5. The resemblance of these pathological changes to those attributed to syphilis is discussed.

6. In our experience no other disease of the fetus produces a similar picture in the placenta.

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DESCRIPTION OF PLATES

Plate 24

Fig. 1. S–37–292. Placenta of the hydropic variety showing great increase in size and gray, deeply fissured maternal surface.

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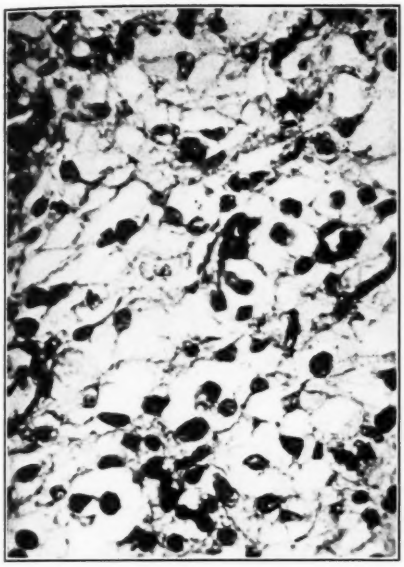
PLATE 25

FIG. 2. S–37–292. Villus from a placenta of the hydropic variety. Great enlargement of the villus is shown. The stroma is of both the hyperplastic and the edematous type. Hematoxylin-eosin stain. × 120.

FIG. 3. Same placenta showing edematous stroma with large multipolar stromal cells and vacuolated Hofbauer cells. Hematoxylin-eosin stain. × 420.

FIG. 4. Same placenta showing hyperplastic stroma with foci of erythropoiesis. Hematoxylin-eosin stain. × 420.

FIG. 5. Same placenta showing vacuolated immature syncytium and persistent Langhans' cells. Hematoxylin-eosin stain. × 420.



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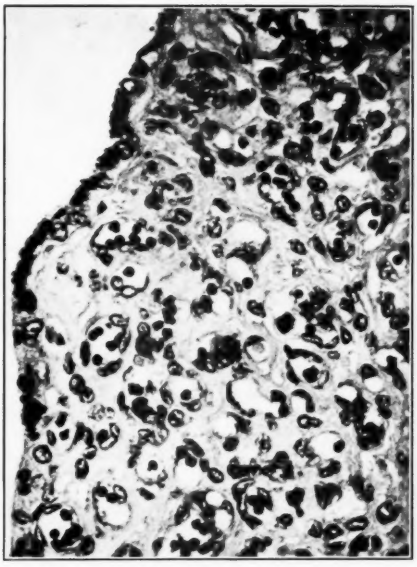

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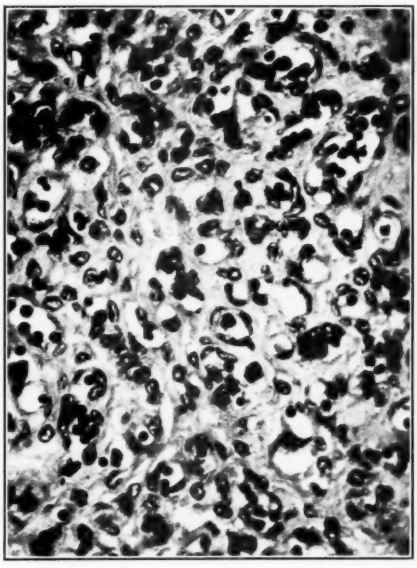

4

Hellman and Hertig

Pathological Changes in Placenta

CALCIFICATION OF THE AORTA, HEART AND KIDNEYS OF THE ALBINO RAT *

KATHARINE PATTEE HUMMEL AND LEROY L. BARNES

(From the Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.)

Reports of the laying down of inorganic salts in the tissues of the ordinary laboratory rat are rare in the literature. Arteriosclerosis is thought not to occur spontaneously, although Hueper [1] has reported that in the course of routine histological examination of 75 experimental rats 12 were found with extensive calcification of the pulmonary arteries. Other organs, such as the aorta and kidney, showed no such change. Duff [2] makes the statement that he has found no independent information concerning the occurrence or frequency of spontaneous arteriosclerosis in rats, and with the exception of hypervitaminosis D, the rat does not respond to the experimental production of arteriosclerosis. Other observations on calcification of the kidneys of the rat have been reported. Polak [3] observed that kidney stones were found in nearly all young rats fed a complete diet with the addition of 2 to 3 per cent calcium carbonate. Eppright and Smith [4] have noted also that histological examination of rats receiving in addition to a basal diet a mineral supplement of calcium and phosphorus revealed extensive calcification of the kidneys.

During the past 6 months numerous instances of calcification have been observed in autopsies of rats in the course of life span studies in the laboratory of animal nutrition, Cornell University. The rats examined were from 3 experimental groups. Lesions were demonstrated in two ways: the aorta, heart and kidneys were X-rayed and then fixed for histological section. Tissues were fixed in Bouin's and Helly's solutions or in alcohol-formalin, sectioned at 10 μ and stained with hematoxylin and eosin or with silver nitrate.

The oldest of the animals belong to the last survivors of a group being studied for the effect of high (20 per cent) and low (8 per cent) protein diets on longevity. Six animals, males and females, ranging from 898 to 1160 days of age, were examined. There was

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no clear and definite evidence of calcification. Therefore, it may be concluded that age is not the most important determining factor in the production of arteriosclerosis.

The second group, which showed almost negative results, was a group of male animals ranging from 261 to 374 days of age, of which 48 were killed and 64 died. Of these, only 2 showed any evidence of arteriosclerotic lesions. The aortas of these animals showed small spots in the X-ray films. None showed calcification of the kidneys. In 8 instances, as will be mentioned later, there was evidence of beginning calcification in the formation of cartilage in the large blood vessels at the base of the heart.

The group of rats that showed a large percentage of calcification were representatives of an experimental group (male and female) designed to show the effect of slow growth on the life span. The general setup of this experiment was similar to that reported by McCay, Crowell and Maynard.[5] Groups I and II represent controls, which received a basal diet adequate in all respects except for calory content, which was fed in addition. The basal diet was identical in composition and amount fed per rat as that in Group III. Group III *a*, *b*, *c* and *d* were allowed only enough of the basal diet for maintenance, and were thus retarded in growth. They were subjected to this procedure 200, 500, 700 and 850 days to date. As seen in Table I, the retarded animals show calcification to the greatest extent.

TABLE I

Frequency of Calcification

	Groups I and II (controls)	Groups III *a*, *b*, *c* and *d* (retarded growth)
Number of animals	8	12
Age in days	662–847	696–862
Calcification		
heart	4	10
aorta	4	12
kidneys	4	12

Examination of the X-rays revealed that in the aorta calcification occurred in two regions: the arch, and the abdominal region at the level of the renal arteries (Figs. 3 and 5). In the heart, calcification occurred most frequently in the large blood vessels close to the semilunar valves and only rarely in the ventricular region. In the kidneys there were found large accumulations in

the papillary region and often a distribution of fine particles over the entire medullary region (Fig. 1).

Histologically the findings in the aorta were similar to those seen in hypervitaminosis D. An accumulation of salts is deposited in the media without, apparently, any previous necrosis of the tissue or deposition of fat particles. The salts are deposited in fine particles along the fibers or in such masses as to make the media appear solid (Figs. 6 and 7). The only other visible change in the aortas is a loosening of the fibers with the appearance of spaces between them. This change, however, has been observed in all aortas of old animals and may be a normal indication of senility, the deposition of calcium being quite unrelated to it.

In the heart, calcification is often preceded by the formation of cartilage in the vessel wall (Fig. 9). This cartilage later becomes calcified. Additional evidence of this procedure is found among the younger rats previously mentioned where of the 48 killed 8 showed the beginning of cartilage formation in the vessels at the base of the heart (Fig. 8).

In the kidneys the calcium is deposited as concretions or stones (Figs. 2 and 4), or in the epithelium of the tubules (Fig. 2). The concretions are found in the collecting tubules near the renal pelvis, blocking the entrance, and often bulging into it. Only occasionally are stones found loose in the pelvis. Calcification of the epithelium of the renal tubules seems to indicate a previous necrosis of the cells.

No explanation can be given at the present time for the differences found in these groups. The nutrition may have been a factor since the diets were designed to permit the same ingestion of essential nutrients such as protein, vitamins and inorganic elements by each individual rat without regard to the body weight. In such experiments the retarded animals ingest more inorganic matter per unit of body weight than the normal growth controls. Further experiments will be necessary to test such a working hypothesis. These diets will be discussed in detail when this study is completed.

Another consideration which can not be disregarded is that of endocrine disbalance. The fact that the type of lesion found in the arteries is similar to that found after treatment with large doses of adrenalin or thyroxin is of interest. It has been shown

(Asdell and Crowell [6]) that in retarded rats there is a definite cessation of sexual activity. This might well be due to a pituitary disturbance manifesting itself in other ways as well.

Summary and Conclusions

1. The aorta, heart and kidneys of the ordinary laboratory rat were examined by X-ray and by histological section for deposition of inorganic salts.

2. Calcification was found in all rats retarded in growth by decreased caloric intake.

3. Age was found not to be the primary determining factor.

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DESCRIPTION OF PLATE

PLATE 26

FIG. 1. X-ray of kidneys, showing distribution of inorganic salts. Rat 21 III *b*, retarded 500 days. × 2.

FIG. 2. Section of kidney showing distribution of inorganic salts. Rat 25 III *a*, retarded 200 days. × 7.

FIG. 3. X-ray of aorta showing calcification in two regions. Rat 21 III *b*. × 2.

FIG. 4. Section of kidney after decalcification, showing a concretion. × 16.

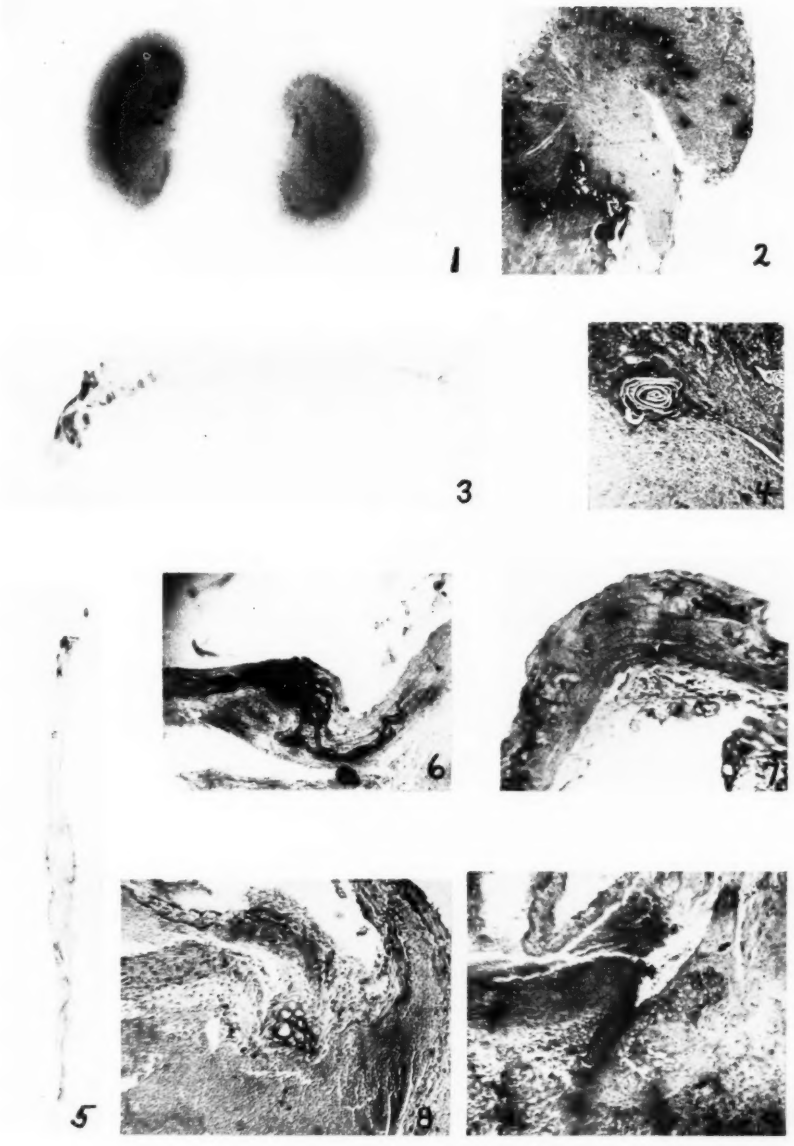
FIG. 5. X-ray of aorta showing calcification along entire length. Rat 60 III *c*, retarded 700 days. Natural size.

FIG. 6. Section of aorta at the base of the heart. Rat 21 III *b*. × 200.

FIG. 7. Section of aorta at arch. Rat 21 III *b*. × 200.

FIG. 8. Section through auricular region of heart showing cartilage formation. Rat k 494. × 100.

FIG. 9. Section through auricular region of heart. Rat 110 II. × 100.



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